THE ECOLOGY AND CONSERVATION OF THREATENED DAMSELFLIES

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by

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Abstract

This thesis presents the results of an autecological study of the Scarce Blue-tailed Damselfly, *Ischnura pumilio* (Odonata: Coenagrionidae) in south-west England. *Ischnura pumilio* has a sparse, localised distribution in the UK, and is classed as "scarce" in the British Red Data Book of Insects. The aim of this study was to bring the ecological understanding of this species to a similar level as that of other threatened odonates such as *Coenagrion mercuriale,* with a view to informing its conservation management. Fieldwork was conducted in the New Forest, Hampshire and at a variety of sites throughout Cornwall and Devon.

Intensive mark-release-recapture (MRR) studies were conducted at two sites during 2005 and 2006. This revealed that despite its reputation as a long range disperser, *I. pumilio* was generally very sedentary with movement characteristics similar to those of other similarly sized odonates. No movement between sites was recorded and 88% of individuals moved less than 50m (net) in their lifetime. Movement distance was inversely density dependent, indicating a tendency to move towards either conspecifics or areas of more suitable habitat. The presence of parasitic mites (*Hydryphantes* spp.) significantly increased movement distance. Males consistently moved further than females and longer intervals between captures resulted in greater movement distances.

Surveys of vegetation and environmental factors were conducted at 31 sites with records of *I. pumilio* from the previous 10 years. The species occurred at sites with a range of water depths, management regimes and levels of pH, grazing, pollution and disturbance. Occupied habitats generally had slow-flowing water, some bare ground at the water's edge and low levels of shade. A low overall odonate species count was also associated with *I. pumilio* presence. Areas away from water were found to be important for the species and over 30% of matings were recorded at least 10m from water. Existing sites should be actively managed up to 25m from water, and new habitat created where possible with dispersal potential in mind.

Survival and recapture rates for *I. pumilio* and *C. mercuriale* were estimated using single and multistate MRR modelling techniques. Resulting rates were used to estimate population size for the *I. pumilio* populations surveyed in 2005 and 2006, and two *C. mercuriale* populations in south England surveyed during 2001 and 2002. Survival generally decreased with age and time in the season and a negative effect of parasites was also indicated. Removing a leg for genetic analysis was found to have no negative effect on survival. The effect of sex was either absent or negligible in mature individuals. *Ischnura pumilio* was found to exist in much smaller populations than *C. mercuriale* and as such may suffer greater levels of inbreeding. A comparison of monitoring methods showed transect walk estimates to be a reliable method of abundance estimation, providing a good basis for further work to develop a predictive relationship.

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Chapter 1: Introduction

This thesis presents the findings of an autecological study of the Scarce Blue-tailed Damselfly, *Ischnura pumilio,* in southern England. The species is classified as scarce in the British Red Data Book of Insects (1987), and in 2005 was considered for priority status in the UK Biodiversity Action Plan. Despite this application being unsuccessful, the species is still a cause for concern from a conservation perspective due to loss of habitat and its tendency for ephemeral occupancy of what habitat remains. The species has a sparse, patchy distribution in Britain and is restricted to southern and western sites.

Effective conservation requires extensive knowledge of a species' ecology, including movement and dispersal capability, habitat requirements and population dynamics. Informed management of habitats may then be undertaken at all landscape scales. Despite the apparent rarity of *I. pumilio,* very little quantitative work on the species has been published and aspects of its ecology which may inform its conservation management are not well understood. Therefore, the aim of this thesis was to bring the level of knowledge about this species to a similar level as that of other threatened odonates such as *Coenagrion mercuriale*, and to provide information which may be used for management planning. Furthermore, some of the techniques employed were applied to existing *C. mercuriale* data in order to add to the body of knowledge about this rare species.

The remaining sections of this chapter consider the rationale for a study of insect conservation with special reference to odonates and review the current literature concerning *I. pumilio*. This is the most comprehensive review of the small body of literature concerning *I. pumilio* (<30 articles; http://www.scopus.com) that has been undertaken to date. Furthermore, this thesis is certainly the first large study of the species conducted and one of very few to include quantitative analyses.

1.1. Conservation of insects and Odonata

Of all the animals and plants on Earth, more species of insect have been described than any other taxonomic group. Over a million species of insect are known to science (Pyle *et al.*, 1981; Thomas, 1994) and several times that number are likely to be as yet undiscovered. They are the largest contributors to biodiversity and provide numerous essential ecosystem services. Yet despite their abundance and variety, the majority of scientific interest in insects has been in controlling those which are detrimental to our health, crops and other food supplies. Attempts to control these pests and vectors of disease have been mostly unsuccessful, and meanwhile many other insect species have declined or become extinct (Pyle *et al.*, 1981). The importance of insects to mankind cannot be overstated. As crop pollinators, insects are vital in food production for the ever expanding human population. Insects produce domestic products such as silk and honey, and provide biological control of the pests of countless crops. They are also the subject of art and literature worldwide, as well as medicinal and evolutionary research which has advanced our understanding of life on earth enormously.

Dragonflies and damselflies are large, beautiful, charismatic insects, which have been admired and collected for centuries. As such they represent a talisman for insect conservation, and may be used to great effect in educating people in the fascinating biology, ecology and behaviour of insects (Switzer, 2007). They are popular subjects for art and photography, and have both global (Worldwide Dragonfly Association) and national (British Dragonfly Society) societies devoted to their study and conservation. Britain has been a leading force in insect conservation for decades, and records held by organisations such as the British Dragonfly Society and Butterfly Conservation have allowed spatial and historical studies to be undertaken (Pyle *et al.*, 1981; Hunter, 2002). However, the trend for submitting records among private recorders is decreasing, which hinders modern ecological studies due to lack of information from recent years (Foster, 1991).

Habitat fragmentation and loss represent a major threat to many insect species (Pyle *et al.*, 1981; Foster, 1991; Hambler & Speight, 1996; Hunter, 2002; Samways, 2007). Odonates are particularly threatened as they are restricted to areas of water for larval development, and wetland habitats have declined by 50% in the UK since 1945 (Nature Conservancy Council, 1984). The draining and conversion to agriculture of wetlands will have severely impacted the distribution and abundance of odonate species. In addition, as habitat becomes increasingly fragmented, populations become isolated, and can lose the genetic diversity which allows adaptation to environmental change (Dieckmann *et al.*, 1999). Odonates are highly vulnerable to pollution of water bodies which is now virtually ubiquitous in the UK in some form.

Insects, including odonates, are increasingly suggested as ecological indicators of changing environmental conditions (Pyle *et al.*, 1981; Chovanec *et al.*, 2004; Bried *et al.*, 2007). The response of the lepidopteran, *Biston betularia,* to industrial pollution has been known and studied for over 50 years (e.g. Kettlewell, 1958; e.g. Daly *et al.*, 2004). Odonates are particularly suitable as indicators of water quality by their presence, abundance and diversity, as they are easy to catch and identify in most cases. The EC Waterframework Directive (European Commission, 2000) requires that all surface water bodies should attain a "good ecological status" in terms of the structure and function of the aquatic ecosystem. Assessment of water bodies is based on biological elements including plants, invertebrates and fish, and may be enhanced by the development of practical bioindicator methods. In response to this the Odonate Habitat Index was developed to provide a sensitive metric to measure biological response to human activities. Due to their specific habitat requirements, odonates were found to represent an ideal indicator or 'umbrella' group for bioindication purposes (Chovanec *et al.*, 2004).

The Odonata are therefore both worthy and desirable targets for conservation research and management. Of the 22,500 species of insect recorded in Britain, odonates (along with butterflies) are among the best recorded but are declining at a rate of 5% per century (Hambler & Speight,

1996). Six of the 40 or so British species are included in the British Red Data Book, including *I. pumilio* and *C. mercuriale*. At the time this work began, *C. mercuriale* was the only British odonate with Biodiversity Action Plan Priority status, though *Aeshna isosceles* was added in 2007.

The behavioural ecology of odonates has been well studied and survival estimates have been published for a range of species using various methods of estimation. However, estimates of population size are rare, and modern methods of population modelling have not been widely applied to odonates. Estimates of survival rates and their influencing factors along with population size estimates and other ecological information allow conservation tools such as population viability analysis to be applied. Reliable population size estimates are important for threatened species, as along with genetic information such as levels of inbreeding, they provide information on the relative ability of populations to adapt to environmental and climatic change, and ultimately avoid extinction.

Previous work on dispersal in another rare damselfly species *C. mercuriale*, has revealed that its limited dispersal capacity is likely to be an important factor influencing its persistence (Rouquette & Thompson, 2007a). Southwood (1977) proposed that species occupying ephemeral habitat such as *I. pumilio* (see section 1.1.4.) should be highly dispersive in order to utilise new habitat as it occurs. The species is reputed to be capable of long range dispersal (see section 1.1.6), but this has not been quantitatively studied before now.

It is essential to effective conservation planning that the habitat requirements of a species are identified, including the extent of the habitat and the resources utilised within it. Without clearly defining what constitutes habitat as distinguished from the surrounding matrix, studies of dispersal between habitats become problematic as well as defining protected areas. The suitability of a site for *I. pumilio* will depend, at least partly, on environmental factors such as water depth, flow rate and pH, which will influence the composition of odonate and vegetation communities. As *I. pumilio* is often over-looked due to its small size and ephemeral habitat use (Dapling & Rocker, 1969; Cotton, 1981; Fox, 1987) a combination of

odonate and plant species composition, along with abiotic measurements, may provide a useful indicator of the likelihood of *I. pumilio*'s presence at a site.

1.2. *Ischnura pumilio* distribution and status

At the end of the 19th century, *Ischnura pumilio* was considered almost extinct in Britain (Lucas, 1900). However, records suggest that historically the species was more widespread than data accounts for, due to the small-scale and transient nature of its preferred habitat. (Fox, 1989; Fox & Cham, 1994). As *I. pumilio* is often found in areas with few other odonates species, and in habitat which appears unfavourable, it seems likely that populations may never have been recorded due to their location (Dapling & Rocker, 1969; Fox, 1987). N. MacNeill (unpub.; as cited in Cotton, 1981) said of *I. pumilio* "Its furtive habits and choice of such sites as the clayey drains may more account for its 'uncommonness' than actual rarity".

In 1987, when official recording of British odonates by the Biological Records Centre (BRC) ceased, Fox published a summary of the habitats of *I. pumilio* in Wales "in the hope that readers will take a second look at all *Ischnura* species, lest *I. pumilio* be further overlooked". He suggested an element of misidentification (confusion with *Ischnura elegans* and other coenagrionids) may have compounded the problem of recording this species, whose status was not then, and is still not, well understood. This was supported by Cotton (1981), who reported that several records of *I. pumilio* from museums were in fact misidentified specimens of *Enallagma cyathigerum* and *I. elegans*. In addition, there were records for which the voucher specimen has been lost, and therefore could not be treated with certainty of correct identification. Fraser (1949) commented "I do not know of any other British dragonfly which has offered so much difficulty in identification or over which so many errors have been made".

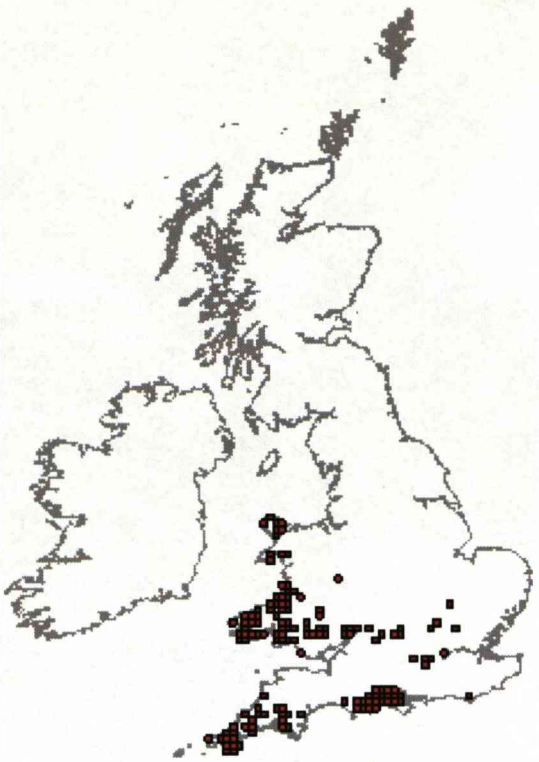


Figure 1.1. Map of the UK distribution of *I. pumilio*. Red squares represent 10km squares in which the species has been recorded. Map taken from NBN Gateway (www.nbn.org.uk/).

I. pumilio is generally considered to be a Mediterranean species (Corbet *et al.*, 1960) and can be found from North Africa, through Europe and the Middle East, to west and central Siberia. It is present across Europe in scattered colonies, but is most numerous in the south (Askew, 1988; Dijkstra & Lewington, 2006). It has been recorded in the Azores, an archipelago of volcanic islands in the Atlantic Ocean (Cordero Rivera *et al.*, 2005), and is also widespread in the mountains of Middle Asia (Borisov, 2006). The species reaches the northern limits of its range in the UK causing its restriction to only southern and western sites (Cham, 1990; see Fig. 1.1). It has been proposed that the distribution of *I. pumilio* is restricted by the February Minimum Temperature (FMT) isotherm, and that it does not occur where FMT is below 2.2°C (Chelmick, 1980; Fox, 1990). In 1991, Cham observed that *I. pumilio* was undergoing an easterly expansion of range, possibly due to habitat creation by quarrying. However, since then the species has been in decline, particularly those recent populations in eastern Britain (Cham, 1991, 1996). In 2005, the species was (unsuccessfully)

considered for UK Biodiversity Action Plan Priority status, and remains a cause for concern from a conservation viewpoint (Daguet, 2005).

1.3. Habitat

Ischnura pumilio is thought to have very specific habitat requirements in Britain which contribute to its apparent rarity (Fox, 1987; 1989). The species is said to be restricted to shallow, base-rich water with a slow flow-rate and to require a degree of openness in the form of bare, muddy ground and sparse vegetation (Fox, 1987; 1989; Cham, 1991; Fox & Cham, 1994). This specific microclimate is inhabited by *I. pumilio* in a wide variety of natural and man-made sites (Jurzitza, 1970; Zimmermann, 1973; Fox & Cham, 1994; Cham, 1996). It has been recorded in natural habitats such as marshy ox-bows, the edges of pools and streams, and in flushes, valley mires and marshes (Fraser, 1941; Fox, 1987). It rapidly colonises newly formed habitat, such as ditches (Dapling & Rocker, 1969), bomb craters, quarries (Cotton, 1981), and wetlands created by clay, gravel, sand, limestone and chalk excavation (Zimmermann, 1973). The species was also recorded in motorway storm-water retention ponds in France, where a high concentration of copper and zinc was present in the top sediment layer and herbicides in the water column (Scher & Thièry, 2005); and has even been observed ovipositing in a puddle by the roadside (Hammond, 1983).

Fox and Cham (Fox & Cham, 1994) analysed records submitted to the Odonata Recording Scheme (ORS) of the BRC, in order to establish habitat use by *I. pumilio* across the UK and determine possible reasons for the continued rarity of the species. They concluded that the important habitat features for *I. pumilio* in the UK were low water velocity, a limited amount of emergent vegetation for oviposition, without the water becoming "choked" with plants (Fox, 1989), and a varying but considerable degree of habitat disturbance. In fact, this species responds exceptionally well to disturbance, including human activity. Numerous colonies have been recorded in areas of mineral extraction, where shallow springs and pools are formed with little vegetation cover, but conditions can be highly unstable

(Fox, 1989; Fox & Cham, 1994; Cham, 1996). In these artificially created sites, colonies rarely persist for more than a few years as vegetation soon encroaches, particularly where water is slow-flowing. However, where there is a continual supply of water and a degree of openness is maintained, persistence is increased (Fox & Cham, 1994). Even such transient populations are of great importance, as although they do not represent a stronghold for the species, they do provide dispersive adults to colonise new areas. Where quarrying is underway this may be especially beneficial, enabling the colonisation of newly mined areas as previous ones become unsuitable.

Although many areas where *I. pumilio* has persisted for many years are those created naturally, there are artificial sites where the species has been recorded for more than 10 years. Disturbance is highly important at these sites, and may take the form of grazing of emergent vegetation and poaching of the substrate by livestock or, somewhat surprisingly, the activities of motor-bike and four wheel drive scrambling (Fox & Cham, 1994). Persistence of *I. pumilio* at these sites is not in spite of such activities, but probably largely because of them as they serve to prevent encroachment of successional plant species, and even create new, small water bodies for the species to colonise. An analysis of all records for the species showed 52% of records came from completely artificial habitats. As most of the "natural" sites in Dorset, New Forest and south Wales are in some way maintained (by grazing, ditching or otherwise) the true proportion will be much higher (Fox & Cham, 1994). This demonstrates the species' dependence on human activity, and its vulnerability to changes in management practices.

Typically, *I. pumilio* colonies in south and west Britain are found in recent artificial water bodies such as industrial reservoirs and gravel pits (Fox, 1989). In Ireland, more than 80% of sites are bogs, pools and seepages on wet heathland, representing the most natural and undisturbed habitat types (Cham, 1996), although the species also inhabits disused quarries in Ireland (Cotton, 1981). Springs and flushes running over mineral substrates are also inhabited by *I. pumilio*, particularly in the New Forest, Forest of Dean and Pembrokeshire (Hammond, 1983; Fox, 1987). Habitats in Wales are often

mesotrophic dew ponds or drinking sites for livestock (Fox, 1987; Fox, 1989). The poaching of the water's edge by animals is a common feature of those water bodies with a more permanent status, and serves to cause enough disturbance to prevent succession of emergent plants. In artificial sites it is their temporary nature which provides the species' requirements for early-successional habitat.

Ischnura pumilio has been found at altitudes above 400m on occasions in Wales (Fox, 1987) and up to 3360m in the alpine regions of Middle Asia (Borisov, 2006). Almost 75% of records from Ceredigion are from 100m or more above sea level, unlike close relative *Ischnura elegans* which is a lowland species in Wales with over 75% of records occurring below 200m. *Ischnura pumilio* was the only species recorded at these high-altitude locations, suggesting that winter temperature is not a major factor influencing its range (Fox & Cham, 1994), and again demonstrating the species' ability to persist in conditions which would appear unfavourable for Odonata.

Ischnura pumilio prefers base-rich water, with higher pH than other damselflies, and is absent from the most acidic habitats such as those dominated by *Sphagnum* (Fox, 1987). Streams occupied by *I. pumilio* are typically base-rich flushes and spring lines, occasionally dominated with *Juncus* rushes, and often shared with *Coenagrion mercuriale*, *Ceriagrion tenellum* (Fraser, 1941), *Orthetrum coerulescens, Pyrrhosoma nymphula, Sympetrum striolatum* and *Cordulegaster boltonii*. In pond habitats, *P. nymphula,* and *S. striolatum* are again common associates of the species, and are often joined by *I. elegans, Enallagma cyathigerum, Coenagrion puella, Anax imperator* and *Libellula depressa* (Fox, 1987). Although excessive plant growth can exclude *I. pumilio*, some emergent vegetation is required for oviposition, usually soft stemmed grasses (Fox *et al.*, 1992). Typical co-occurring plant species include *Juncus* species, *Typha* species, *Agrostis stolonifera*, *Glyceria fluitans* and *Ranunculus flammula* (Fox, 1987, 1989, 1992). It is thought that tenerals and adult females take shelter in thick scrub formed by bog myrtle (Fraser, 1941).

A cytological genetic analysis (Kiauta, 1979) revealed the karyotype of *I. pumilio* to be 2n = 29, n = 15. The author noted that this n complement is higher than the 12 other members of the genus analysed and than the coenagrionid type number; n = 14. This was related to the unusual ecology of *I. pumilio*, in that it can colonise new and ephemeral habitats, where it remains for only a few years. The increased recombination index resulting from a higher chromosome number infers a greater scope for genetic variation, which could explain the observed ecological flexibility of the species. This theory is supported by four other coenagrionid species with high n complements, which also have peculiar ecological adaptations (*Argia apicalis, A. tibialis, E. cyathigerum,* and *Leptagrion macrurum*; Kiauta, 1979). In contrast, *C. mercuriale* has a unusually low karyotype of n = 13 and low recombination potential (Kiauta & Kiauta, 1988). This was suggested to be the reason for the species restriction to highly specific habitats and inability to adapt to environmental variation.

1.4. Life history

Female *I. pumilio* are unaccompanied by the male during oviposition and the insertion of each egg takes approximately 20 seconds. Various species of soft stemmed plant are used by females for oviposition, including *Juncus inflexus, J. articulatus* and *Eleocharis palustris.* Where equal densities of these plants were present, over 75% of females were observed to oviposit in *J. articulatus*, indicating females may prefer certain species when several are present (Cham, 1992a). Females lay eggs down a stem until the thorax makes contact with the water, but no attempts to submerge fully have been recorded, possibly due to the problems of overcoming surface tension. The species' ability to persist in areas prone to drying out in summer may be due to egg laying in particular parts of the stem where eggs become entombed by plant tissue, and are therefore protected from desiccation (Cham, 1992a).

Ischnura pumilio is primarily a univoltine species (Cham, 1993), although not all captive larvae complete their life cycle within a year (Fox & Cham, 1994). This short generation time may be advantageous given the transient

nature of its chosen habitat (Fox & Cham, 1994), allowing full development of a cohort before major changes take place. Emergence is highly synchronised, both in the wild (Fox, 1990; Cham, 1993; Cordero Rivera & Andrés, 1999), and in the laboratory (Cham, 1992a). Under laboratory conditions 90% of prolarvae emerged on one morning, after 17 days at 25°C, with the remainder following the next day. When subjected to drought conditions for the duration of the hatching period, prolarvae emerged two days after resubmergence of the plant (Cham, 1992a).

Following hatching, the prolarval skin splits and the second instar larva emerges. Within minutes these larvae can be observed to use threat displays to each other, which appear to encourage dispersal away from the overcrowded stem (Cham, 1992a). This is unusual among the Zygoptera, as in other species threat displays have only been observed from the fourth instar onwards (Rowe, 1985). Early dispersal may be advantageous in shallow water conditions, as in the event of drought, some larvae will survive in areas which remain wet (Cham, 1992a).

An accurate method for identifying *I. pumilio* larvae has been an issue of some debate. One method can be obtained by combining features of the method outlined by Miller (1987), with the amendments listed in Cham (1992b). Previous methods such as those described by Fraser (1949) and Carchini (1983) were found to be ineffective by Cham (1992b), as "numerous exuviae and larvae known to be *I. pumilio* have consistently keyed out to *I. elegans*". No published studies of the ecology of larval *I. pumilio* exist and this may be due to the difficulty of identifying larvae and of dredging them from aquatic vegetation (Fraser, 1949).

Male *I. pumilio* are non-territorial and engage in scramble competition for access to mates. A study of lifetime mating success (LMS) revealed that a large proportion of males never mate (43.6%, n=142) compared with a small number of females (13%, n=100; Cordero Rivera & Andrés, 1999). Variance in LMS was greater in males, with one male obtaining the maximum of 7 matings, whereas the maximum for a female was 4. Individuals with longer lifespans achieved the most matings, but body size had no significant effect on LMS in either sex. Copulation duration

averaged 1 hour 19 minutes, but one pair was observed in copula for approximately 5 hours. Copulation duration was negatively correlated with time of day.

It has been reported that *I. pumilio* do not mature near water, but rather appear in large numbers as mature adults. A mark-recapture study failed to find one teneral specimen, despite daily sampling near water where mating *I. pumilio* had been observed the previous year, and teneral *Ischnura graellsii* were common (Cordero Rivera & Andrés, 1999).

The female of *I. pumilio* has two colour forms, the immature or '*aurantiaca*' form is bright orange, and is observed in its highest numbers at the beginning of the flight season. The mature, sexually-active adult form is pale yellow-green (Cham, 1990) and has an apple-green ventral surface, which is clearly visible *in copula* (Kyle, 1961). It is only this fully-developed female form which has large mature eggs (Kyle, 1961; Zimmermann, 1973). In some observation studies, *aurantiaca* females have not been observed in copula and appeared unattractive to males (Kyle, 1961; Cham, 1990). Other studies have observed male attraction to this form, resulting in vigorous attempts to repel males by early-stage *aurantiaca* females, and some copulation by late-stage females (Jurzitza, 1970; Reinhardt, 1990; as cited in Langenbach, 1993), suggesting that sperm can be stored until eggs are mature (Cham, 1993). However, *aurantiaca* females have never been observed ovipositing (Langenbach, 1993).

Male *I. pumilio* are smaller than females (Cordero, 1994), have a black thorax with green or blue sides, and a small patch of vivid, azure blue covering segments eight and nine of the otherwise black abdomen. The immature form is also differently coloured to the mature form, in that abdominal segments 8 and 9 are coloured brown (Cham, 1990; Cham, 1993) or yellow-grey (Kyle, 1961) which lasts less than 48 hours before developing signs of blue. However, three captive-reared males were observed to emerge as orange, *aurantiaca,* by Kyle (1961), changing to an intermediate yellow-grey stage within 24 hours, and becoming typical adults with dark abdomen and blue tail within a further 24 hours. Kyle suggested that these males were atypical in their initial colouration, and this was

confirmed by the observations made during the fieldwork for this thesis, where all males conformed to Cham's description. Occasionally, intermediates of the immature and mature colour forms have been observed in both males (Kyle, 1961; Cham, 1993) and females (Cham, 1990; 1993), but the rarity of these in males indicates that colour change is rapid.

Female maturation takes somewhat longer than male, with the *aurantiaca* stage lasting 5-9 days, the intermediate stage 2 days, and the mature female living up to 26 days further (Cham, 1993). In a study using outdoor-captive-reared females, a similar 6-12 days was observed for maturation, and colour change again usually took 2 days (Langenbach, 1993). The latter study indicated that maturation time was inversely proportional to food supply and temperature. However, date of emergence was most highly correlated with maturation time: later emergence resulting in shorter time to colour change. Cordero (1994) found insectary-reared females had a significantly longer total adult lifespan (emergence to death) than males (means ± SEM and maxima: females = 16.3 ± 1.94 and 37 days; males = 10.7 ± 1.02 and 26 days) with one hand-reared female excluded from the analysis surviving for 57 days. In contrast, field studies show female lifespan to be less than that of males, but it has been suggested that no real difference exists, and that the perceived difference is due to the increased likelihood of recapturing males to females in mark-recapture studies (Cordero Rivera & Andrés, 1999).

Female *I. pumilio* also exist in an andromorph (or heterochromic) form, although it is generally rare (Langenbach, 1993), and has not been reported at all in Great Britain (Seidenbusch, 1995; Cordero Rivera & Andrés, 1999). An observational study in Germany (Seidenbusch, 1995) revealed that andromorph females were always less abundant than gynomorphs, and were only found in 6 out of 10 sites visited. Andromorph females undergo one extra colour change than gynomorphs, as they first change from *aurantiaca* to the typical green, and then from green to the azure blue typical of males. These two colour changes are often merged, and individuals can be found exhibiting all three colour types. Whilst green females can be identified as future andromorphs by the degree of melanism on various abdominal segments (detail in Seidenbusch, 1995), it is currently not possible to

distinguish the ultimate phenotype of *aurantiaca* females. A mark-recapture study found no significant differences in fitness correlates between morphs (Cordero Rivera & Andrés, 1999).

1.5. Flight period

Records from the BRC database, show *I. pumilio* has been observed on the wing from May to September. In general however, the flight season appears to commence in early June and peak in July, with a gradual tail-off into September at the latest (Fox, 1990; Smallshire & Swash, 2004). More specifically, in Wales flight commences in the last week of June and continues into mid-July, with a few remaining until early August and 68% of dated records coming from the period 20 June to 30 July (Fox, 1987). In south-west England (Cornwall, Devon, Dorset, Somerset, Wiltshire, Gloucestershire, Worcestershire and Herefordshire) a similar number, 63%, of records are from this period (Fox, 1990). Records from Wales (Kyle, 1961) and Bedfordshire indicate the flight season can commence as early as May (Fox, 1987; Cham, 1993). The timing and duration of the flight period in *I. pumilio* is not influenced by habitat (mineral extraction sites, flushes, pools etc) or by geographical area within the UK, except that it may extend slightly later in the south. It is however influenced by altitude, with the earliest emergence occurring at the lowest altitudes and also the longest persistence at the end of the flight season (Fox, 1990).

1.6. Dispersal

One of the reasons for the lack of knowledge about *I. pumilio* is the transient nature of its colonies. Askew (1988) noted that the species will "rapidly colonise newly-formed biotopes, but colonies tend to die out after a few years", indeed it is often found in locations far from the nearest known colony. This suggests that *I. pumilio* has the means to disperse over some considerable distance, which would seem in discord with the weak, erratic flight normally observed (Fraser, 1941; Fox, 1989). However, a potential method for long range dispersal was observed, when on several occasions

mature, adult, male individuals exhibited strong upward flight, to an altitude at which they were no longer visible through ×8 binoculars (Fox, 1989; Cham, 1993). These observations all occurred on clear, still and very hot days which would allow strong thermals and upward currents. It has been proposed that individuals use these to facilitate strong, upward flight leading to dispersal, perhaps aided by directional currents in the air above significant barriers such as trees and buildings. Even weak gusts of wind are sufficient to carry individuals at great speed when flying close to the ground (Dapling & Rocker, 1969), therefore stronger gusts at higher altitude may indeed be sufficient for long range dispersal. Individuals flying close to ground level often fly in the direction of the wind, presumably utilising its force to assist flight, and this has been proposed as a potential mechanism for the observed movement of colonies from year to year (Dapling & Rocker, 1969). It seems highly possible then, that individuals disperse in this manner using the stronger air currents at higher altitudes. Such strong, purposeful dispersal is unusual among sexually mature damselflies, and may provide an opportunity to reproduce before the uncertain outcome of dispersal, (Fox, 1989). A similar strong directed flight was observed by Cham (1993) along the path of a water filled seepage, which may also be a method of dispersal flight.

1.7. *Coenagrion mercuriale*

Coenagrion mercuriale is a conservation priority among European odonates, and is protected by law at a national and continental level (Wildlife and Countryside Act 1981; Bern Convention; European Community Habitats Directive). It is listed as rare in the British Red Data Book of Insects (Shirt, 1987), and until 2008 was the only odonate given priority status in the UK Biodiversity Action Plan (HMSO, 1994; 1995). The species requires one of two increasingly fragmented habitat types in the UK: shallow, permanently-flowing, small streams and channels found in lowland heathland streams over base-rich substrates, or calcareous streams in water meadow systems (Rouquette & Thompson, 2007a). The species is also found in two fen sites in Oxfordshire and Anglesey.

The species has been well studied in the UK (e.g. Strange, 1999; e.g. Purse *et al.*, 2003; Watts *et al.*, 2004; Rouquette & Thompson, 2007a) and several mark recapture studies have been undertaken (Purse *et al.*, 2003; Thompson & Watts, 2004; Rouquette & Thompson, 2007a). This thesis does not aim to extensively review the existing literature on *C. mercuriale*, but rather to contribute to existing knowledge. Previous studies have found that *C. mercuriale* is extremely sedentary (Purse *et al.*, 2003; Rouquette & Thompson, 2007a) and has highly specific habitat requirements (Thompson *et al.*, 2003; Rouquette & Thompson, 2005). Genetic and demographic studies have confirmed that populations are declining and that significant loss of genetic diversity is occurring within them, a problem which is compounded by the species poor ability to disperse between sites (Watts *et al.*, 2004; 2007a). Levels of inbreeding in some isolated populations are comparable to those observed in threatened mammals (Watts *et al.*, 2005). Lifetime mating success in *C. mercuriale* was found to be strongly influenced by climatic conditions, which has implications for the persistence of colonies in the current period of unpredictable climate change (Purse & Thompson, 2005a).

However, estimates of survival and recapture probabilities are lacking for *C. mercuriale*. Therefore, the methods applied to *I. pumilio* in this thesis were also applied to existing data from mark-release-recapture studies of *C. mercuriale*, in order to produce this information for future conservation initiatives. Estimates of population size in odonates are generally rare, although previous attempts have been made for *C. mercuriale* (Thompson & Watts, 2006; Watts *et al.*, 2006). However, they were subject to assumptions which may not be met in field populations of odonates and more reliable methods are required. Accurate population size estimates are important for rare species, as when combined with estimates of genetic variation, they provide an insight into the relative ability of populations to adapt to changing conditions such as weather and habitat. Comparison of the resulting estimates to those produced for *I. pumilio* may provide further insights into the conservation status of these rare species, which belong to

the same family (Coenagrionidae), have similar habitat requirements and often inhabit overlapping areas within a site as adults.

1.8. Thesis overview

This thesis presents the results of a study of the autecology of *I. pumilio* in south-west Britain. Specifically, dispersal and movement capacity, habitat requirements, survival rates and population size estimates are considered. The findings provide the basis for future ecological studies of this species and for informed conservation management and monitoring programs. In addition, population size and survival are considered in *C. mercuriale*, in order to provide further information for the conservation of this rare species. The following chapters are organised as described below.

Chapter 2 presents the results of two mark-release-recapture (MRR) studies conducted in the New Forest during summer 2005 and the Red River Valley, Cornwall in summer 2006. It reports numbers marked and recaptured and patterns of movement with reference to dispersal potential. This includes net and gross lifetime movement and the effect of factors such as age, sex, weather and density thereon. An analysis of the direction of movements and the effect of wind direction is presented. The dispersal potential of *I. pumilio* in relation to other odonates and its own reputation as a long distance disperser is also discussed. Dispersal is a universally important aspect of a species' life history and is of key importance to understanding ecological processes. A species' ability to move determines metapopulation dynamics, maintenance of genetic variation for adaptation to environmental change, as well as predator evasion and mating success. It is therefore crucial to understand a species' dispersal potential if a conservation effort is to be made.

Chapter 3 describes the habitat requirements of *I. pumilio* identified during a detailed study of 31 sites across Cornwall, Devon and Hampshire in 2006. The use and importance of different areas within that habitat are considered, with reference to conservation of habitat away from the immediate water area. Associations between other odonates and *I. pumilio* are presented,

along with the environmental variables influencing these associations. The species is thought to have specialised habitat requirements in the UK, but is found in a variety of habitat types, at a variety of altitudes and levels of human disturbance. In order to avoid further decline of this species, an understanding of the important features of its habitat must be developed and sites preserved in a suitable condition. It is also important to identify the extent of what constitutes habitat for a species. Habitat use may change with daily variation in weather and at night, but all areas must be considered when designing management plans.

In Chapter 4, the results of population modelling techniques applied to the 2005 and 2006 *I. pumilio* MRR data sets are presented. Estimated survival rates are reported, and the variation therein due to sex, age, state of maturity and parasitism is discussed. Population sizes estimated using these methods are also reported, and compared to those more traditional estimates such as transect walk counts and minimum number alive. Although many MRR studies of damselflies have been conducted previously, this is the first to apply multistate modelling techniques and is also the first to use these techniques to consider the effects of parasitism on survival, in damselflies or otherwise. The aim of this study was to determine the rates of survival and recapture in *I. pumilio* and their influencing factors in order to inform conservation management and future study design. Differences in survival between groups within a population and the effect of external factors on survival rates can provide valuable information about how conservation management should be directed.

Chapter 5 reports the results of survival analyses and population size estimates for *C. mercuriale,* performed using the methods applied to *I. pumilio* in Chapter 4. Data from two previous MRR studies of *C. mercuriale* were analysed; these were collected in the Itchen Valley during summer 2001 and at Beaulieu Heath, New Forest in 2002. The effects of sex, time and age are again considered, and the findings compared to those reported for *I. pumilio*. Observed and estimated sex ratios for both species are also presented and discussed with reference to previous studies of sex ratio in odonates.

In Chapter 6, the findings of the previous chapters are considered together and overall conclusions are discussed. The management and conservation outcomes of this research are outlined and potentially informative areas of further research are suggested.

Chapter 2: Movement patterns of adult *Ischnura pumilio*

2.1 Introduction

Movement is a universally important aspect of a species' life history and is of key importance to understanding ecological processes at all spatial scales. From landscape-level effects on metapopulation dynamics, to providing a source of genetic variation for evolutionary change within populations, and individual-level effects such as predator evasion and mating success; there are few aspects of a species' ecology that are not affected by its ability to move (Dieckmann *et al.*, 1999). It is therefore crucial to understand a species' movement potential, particularly in a dispersal context, if a conservation effort is to be made. However, despite its importance, dispersal is perhaps the least well understood life history trait (Clobert *et al.*, 2001).

Natural habitats are generally declining and becoming increasingly fragmented and 50% of the UK's wetlands have been lost since 1945 (Nature Conservancy Council, 1984). Fragmentation causes populations to become more isolated, and this can lead to loss of genetic diversity due to inbreeding, and ultimately extinction. Isolation may also cause the loss of genes for dispersal from the population, as dispersing individuals are less likely to settle in suitable habitat and are therefore subject to increased mortality rates (Dumont & Verschuren, 1991; Dieckmann *et al.*, 1999; Watts *et al.*, 2004).

Ischnura pumilio is classed as nationally scarce in the British Red Data Book of Insects (Shirt, 1987). At the end of the 19th century the species was considered almost extinct in Britain (Lucas, 1900). Despite some recent range expansion (Cham, 1993), it remains an endangered insect due to its specific, early-successional habitat requirements and the threats to suitable wetland habitat. The species reaches the northern limits of its range in the

UK causing its further restriction to only southern and western sites (Cham, 1990).

Little is known about the movement and dispersal characteristics of *I. pumilio*, and one of the reasons for this is the transient nature of its colonies. Individuals rapidly colonise newly formed habitat and are often found in locations far from the nearest known population, but colonies tend to die out after a few years (Askew, 1988). This suggests that *I. pumilio* has the means to disperse over some considerable distance, and has earned the species a reputation as a "wandering opportunist" (Fox, 1989; Cham, 1996). This seems in discord with the weak, erratic flight normally observed (Fraser, 1941; Fox, 1989), which is said to be strongly influenced by even weak gusts of wind when flying close to the ground (Dapling & Rocker, 1969).

A strong, upward flight has been observed in mature, adult males (Fox, 1989; Cham, 1993), on clear, still and very hot days. It was proposed that individuals use thermals and upward currents on such days to facilitate strong upward flight leading to dispersal, perhaps aided by directional currents in the air above significant barriers such as trees and buildings. Individuals flying close to ground level often fly in the direction of the wind, presumably utilising its force to assist flight, and when occurring at higher altitude this may be a potential mechanism for the observed movement of colonies from year to year (Dapling & Rocker, 1969).

Due to the small and transient nature of most of the UK populations of *I. pumilio*, the primary study site was chosen as one of the best in the UK. This allowed a large scale study with a guaranteed large population. As a comparison to this, the same method was followed at a very different area the following year, where populations were much smaller, and formed a potential metapopulation structure. The habitat in each site was also very different, allowing a more broad understanding of the movement characteristics of this species in different circumstances to be formed. When investigating study areas for this project, the two chosen were those with the largest area potentially inhabited by the species, to reduce the constraining

effect of study size on recorded distance (Schneider, 2003; Winkler *et al.*, 2005).

A positive relationship has been found between size and maximum dispersal distance in actively dispersing organisms (Jenkins *et al.*, 2007). Previous studies of odonates have found that dispersal probability increased with increasing species size, with larger species more likely to disperse larger distances (Conrad *et al.*, 1999; Angelibert & Giani, 2003). Damselfly species similarly sized to *I. pumilio* in those studies were mostly extremely sedentary, with a few individuals moving much greater distances (e.g. Angelibert & Giani, 2003; Purse *et al.*, 2003; Rouquette & Thompson, 2007a). As *I. pumilio* is the smallest British odonate, it may therefore be expected to have the smallest dispersal probability. However, its reputation as a long range disperser suggests that it may not conform to this pattern. This chapter will add *I. pumilio* to the list of species already considered and examine its position within this relationship.

There have been relatively few studies of movement and dispersal in odonates and no direct studies of *I. pumilio*. This chapter aims to bring the level of understanding of the movement potential of this species to a similar level to that of other rare odonates such as *C. mercuriale.* This will allow more informed management of the species and its habitats. Specifically the following issues are considered:

- Means and maxima of individual and lifetime movement distances
- Patterns of movement within and between sites
- Effect of various factors on movement such as age, sex, weather and density
- The direction of movements and the effect of wind direction
- The movement potential of *I. pumilio* in relation to other odonates and its own reputation as a long distance disperser.

2.2 Methods

2.2.1 Study sites

The primary study area is located in the New Forest (designated a National Park on 1st March 2005), southern England (SU193112). *Ischnura pumilio* is found on springs and flushes running over mineral substrates in the New Forest (Hammond, 1983; Fox, 1987), and within the study area there are several valleys, each providing suitable areas of this inherently patchy habitat. The British Dragonfly Society database has over 300 *I. pumilio* records from at least 60 locations in the New Forest between 1904 and 2003, including several sightings in the adjacent valley to the north of the main study site. The study encompassed all of the known, potential habitat patches within an area covering approximately 5km × 4km (Fig. 2.1). This area was centred on the largest population within the New Forest. The site inhabited by this population comprised one side of a small valley; where spring-fed flushes run down to form a stream, Latchmoor Brook. There are several patches of suitable habitat within this valley, although from a movement perspective, and for the purposes of this study, the majority can be considered continuous habitat as there are no apparent significant barriers.

The study was repeated the following year in an alternative habitat type. The second study considered a complex of four sites, located along the Red River valley, Cornwall, England; within an area covering approximately 4km × 2km (Fig. 2.2). From west to east these sites are Bell Lake Marsh (SW621418), Rosewarne (SW645417), Roscroggan (SW651420) and Great Wheal Seton (SW655418). The largest of these sites, Great Wheal Seton is approximately 200m by 50m and can be considered the stronghold of the local population. The smallest site, Roscroggan, is approximately 80m by 50m. The species had been recorded at all of the sites in the previous 10 years, and the habitat remained mostly unchanged and within the range of habitat types described in Chapter 3. All four sites were therefore assumed to be potential current sites with a potential metapopulation structure, and at

least likely to be visited by any wandering individuals. Each site formed a distinct patch of habitat, separated by areas of sub-optimal habitat made up mainly of rural-residential and agricultural land. The maximum straight-line distance between a pair of sites was 3.5km and the minimum 0.4km. The whole area was once heavily industrialised (Fig. 2.3), and the pools and marshes inhabited by *I. pumilio* have resulted from the flooding of pits and shafts previously used in tin-mining.

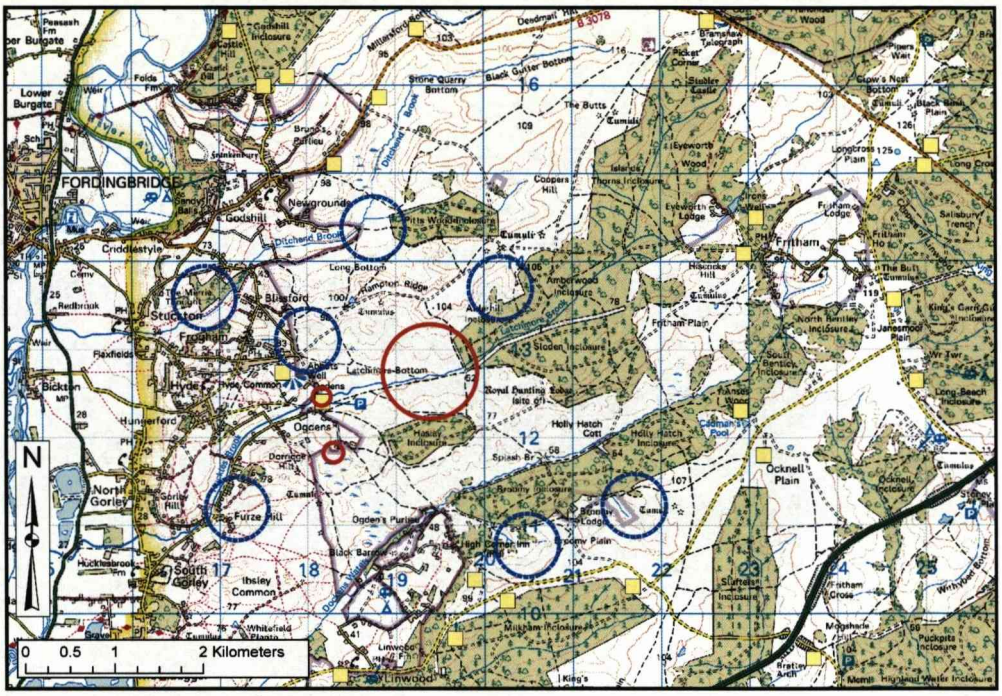


Figure 2.1. Locations surveyed during 2005. Latchmoor Bottom, the main study site is indicated with a large, red circle. Other locations where individual *I. pumilio* were captured are indicated with small, red circles. Blue circles indicate areas which were searched unsuccessfully. Yellow squares indicate car parks where posters were placed (see text).

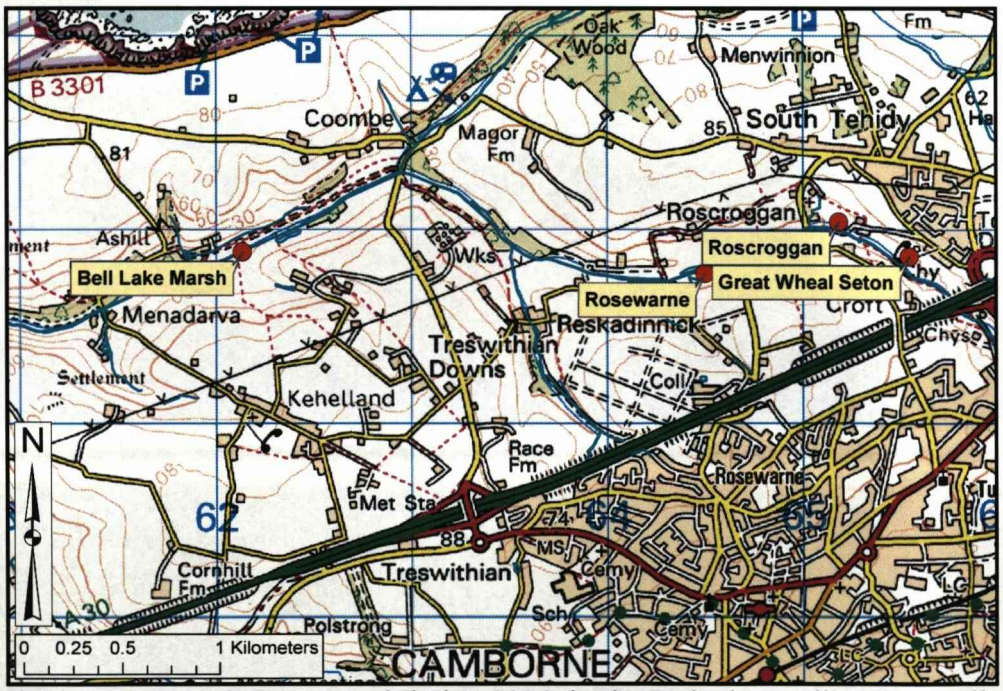
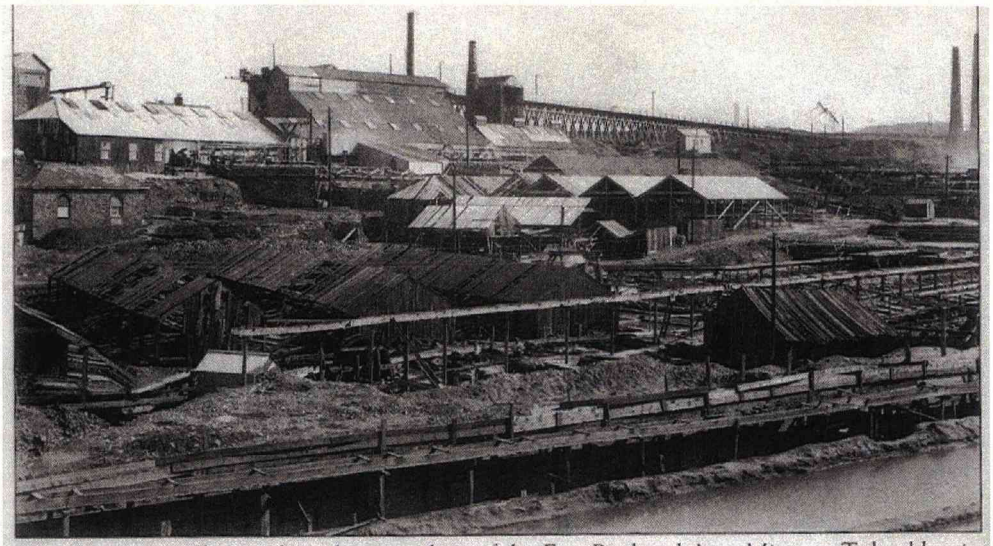


Figure 2.2. Locations surveyed during 2006, in the Red River valley, Cornwall. The four main study areas are indicated with red circles.



The extensive milling and tin dressing plant of the East Pool and Agar Mines at Tolvaddon in the Tuckingmill Valley. The small electrically hauled ore trains ran along the wooden viaduct

Figure 2.3. Above: an illustration of the level of industrialisation at Great Wheal Seton during the period *c.* 1900 – 1930. Below: Great Wheal Seton in 2006.

2.2.2 MRR study

In 2005, a mark-release-recapture (MRR) study of the New Forest stronghold population at Latchmoor was conducted by four field assistants, for 8 weeks commencing 4th June. In 2006, the second area was surveyed following the same method for 4 weeks commencing 3rd June. A pair of field assistants was assigned to half of the study area each day (pairs and

areas were changed randomly each day), and walked around their area repeatedly ensuring that equal time was spent in all parts of the habitat, to avoid bias towards any particular location or habitat type. All *I. pumilio* encountered were caught using a fine-mesh net, and location recorded using a Global Positioning System (GPS) calibrated to the British National Grid.

Every damselfly captured was uniquely marked using an alphanumeric code written on the left forewing using a non-abrasive, waterproof pen (Staedtler Lumocolor 313), and a small dot of coloured paint on the thorax using a non-toxic paint marker pen (Uni Posca PC-3M). Codes were designed for ease of reading, for example certain letters were not used due to their similarity to numerals. This code was recorded each time the animal was recaptured or sighted, to allow the tracking of its movements and activities.

Along with the unique code assigned to the individual, a record was made of the following information: GPS location, sex, wing length (measured with digital callipers), number of ectoparasites (*Hydryphantes* sp.), behaviour and state of maturity as indicated by colouration. Status was recorded as either Teneral (newly emerged, wings not yet dry, colours undeveloped); Immature (females orange, blue of abdominal segments 8 and 9 not fully developed in males); Intermediate (females only, beginning to turn green with orange still visible); Mature (adult colouration fully developed). Behaviour was recorded as Perching, Flight, Tandem, Copula, Oviposition, Harassment (male – female), Aggression (male – male) or Feeding. In addition to recording ground-level flight behaviour, field assistants were briefed to look for and record any upward flight behaviour such as that observed by Fox (1989) and Cham (1993). Teneral individuals were not captured and marked as they are very delicate during this drying and hardening period, and can be identified by the characteristically shiny wings. They were, however, recorded as completely as possible on datasheets. Once an individual had been processed it was returned to a plant stem near the point of capture. The legs were allowed to grasp the stem before releasing the animal, as this helps to prevent immediate flight away from the captor, which may result in emigration.

Locations outside of the study site were searched periodically, by one or two field assistants. These searches were made on, and immediately after, the hottest days, as much large scale dispersal is reported during such weather (Longfield, 1948; Fox, 1989; Cham, 1993), which is probably required to achieve the body temperatures required for such sustained activity. In addition, posters were placed in 20 New Forest car parks within the area (radius *c.*10km) surrounding Latchmoor Brook (Fig. 2.1). These car parks are frequented by walkers, bird-watchers and other naturalist users of the National Park. The posters showed a marked *I. pumilio* individual and requested that any sightings by the general public be reported to a mobile telephone number.

2.2.3 Statistical Analysis

Distances moved were calculated using GPS data, as the straight line distance between relevant points of capture. Multiple captures of an individual on one day were omitted excepting the initial capture. The movement parameters to be estimated (modified from Rouquette & Thompson, 2007a) are given in Table 2.1. Geometric means were calculated for distances as they are a better summary of highly skewed data than the arithmetic mean, and are calculated as the n^{th} root of the product of the data, where n is the sample size (Crawley, 2005).

Table 2.1 Movement parameters calculated from GPS data.

Parameter	Definition
d	Distance moved between successive captures (m)
t	Time between successive captures (days)
T	Time between first and last capture (days)
N	Net lifetime movement (m); distance between first and last captures
G	Gross lifetime movement (m); sum of *d*s for each individual
R	Range (m); maximum distance between 2 capture points

The age in days of each individual was estimated according to the following criteria (negative ages indicate age before maturity and age 1 can be thought of as the first day of maturity).

- Females caught when intermediate were assigned to age 0 as they were about to reach maturity. A female captured multiple times when intermediate was assigned to age 0 on the last occasion.
- If the female was not captured when intermediate, the first capture with mature adult colouration was assigned to age 1.
- If the female was recorded during the immature stage only, the last day of capture with orange colouration was assigned to age -2.
- Males caught when immature were assigned to day -1 and otherwise to day 1.

The criteria were used in the order listed and only one was applied. The duration of each state of maturity used to develop these criteria were taken from Cham (1993). This correction is thought to provide a more accurate estimate of age than simply days since first capture.

To investigate within site variation in movement patterns, the 2005 site was divided into theoretical 10m × 10m grid squares. The scale of sections represents the finest resolution possible given the accuracy of the GPS (5m). Sampling unit size should be 2 to 5 times smaller than the feature of interest, large enough to contain more than one individual, but not so large that there is too much within-unit variability or that the smallest scale cannot be detected (Fortin & Dale, 2005). Thus, the size of each section reflects between a fifth and a half of the approximate scale of lifetime movement for the majority of individuals (see results). Differences in distance moved from each section were tested using a one way ANOVA. Density was then calculated as the number of captures within each square per day of sampling.

A multiple regression was performed to investigate the effects of the following variables on *d*: sex; age (at midpoint of movement); wing length (as an index of size); date (calculated as time since first day of study period

(day 0) to midpoint of movement); time *t*; number of parasitic mites (as counted on capture prior to movement); density of individuals in start locations and weather variables during the period of movement. Only one movement per individual was included in this analysis and the movement selected was the first as a mature adult. This was defined as the movement during which the individual was age 1 or above for at least 50% of *t*. Those individuals without a movement fitting this criterion were excluded from the analysis. Multiple regressions were also performed to investigate the effects of the following variables on net lifetime movement (*N*), gross lifetime movement (*G*) and range (*R*): sex, wing length, lifespan (*T*), first day of maturity (the date an individual was age 1), presence or absence of mites during lifetime and density of individuals in start locations.

Stepwise backwards elimination was used to select significant variables, using a combination of AIC and *F* tests to assess the significance of removing each term from the model (Crawley, 2005). All analyses were carried out on $\log_{10}$ transformed distances as the data were highly skewed (Fig. 2.4). The following variables were also $\log_{10}$ transformed for the same reason: time (*t*), age, date, number of mites and average rainfall. All analyses were performed using R 2.4.0 (R Development Core Team, 2005) and package CircStats for circular statistics (S-plus original by Ulric Lund and R port by Claudio Agostinelli, 2007).

Detailed weather data for the study periods was obtained from local Met Office weather stations at Ibsley (British national grid reference SU162094) and Hurn (SU117978), including hourly temperature, rainfall, light intensity, cloud cover, wind speed and wind direction (UK Meteorological Office, 2006). Examination of weather data showed collinearity between the variables. Consequently, to examine the effect of weather generally rather than the relative contributions of temperature, wind and rain, a compound measure of weather derived from four different measures (average maximum temperature, overall minimum temperature, average rainfall and average wind speed) was used. A principal components analysis was performed on the four measures, and the value of the first two principal

components for each individual was used in the multiple regression (Graham, 2003; R. Knell, pers. com.). The first two principal components accounted for 67% of all the variance in the four measures of weather.

The direction of each movement was calculated using the Easy Calculate 5.0 for ArcGIS extension (Tchoukanski, 2004). Linear directional means and circular variances were then calculated for the direction and orientation of first mature movements in order to examine patterns in the direction of movements and consider ecological explanations including relationship to wind direction.

In order to consider the dispersal probability of *I. pumilio* in relation to other odonates, an adaptation of the method adopted by Conrad *et al.*, (1999) was used to produce a comparable graph. They analysed individuals transferring between ponds as a percentage of all movements (transfers and non-transfers) recorded in an MRR study, which they termed dispersal probability. An approximation of this method was obtained by dividing the site at Latchmoor into four sub-sites. The sub-sites formed distinct population centres with a much higher density of individuals than the surrounding areas (Fig 3.4). Although movements between sub-sites are not strictly dispersal events, they are termed as such for the purposes of this analysis and for comparison to the results of Conrad *et al.* (1999).

Conrad *et al.*, (1999) used an ANCOVA model to test for differences in mean dispersal probability among the main study species; therefore a regression model was used here for *I. pumilio*. Movements transferring between sub-sites were allocated to 100m distance categories and the percentage of recaptured animals dispersed in each distance category was calculated. Conrad *et al.*, (1999) found that dispersal probability decreased exponentially with distance, so percentages dispersed were $\log_e$ transformed for comparability. Only the first transfer movement made by an individual was used, regardless of whether they made subsequent transfer movements. The percentage of recaptures which dispersed ($\log_e$) was regressed against distance dispersed and predicted values from the resulting model were back-transformed and used to plot a curve for *I. pumilio.*

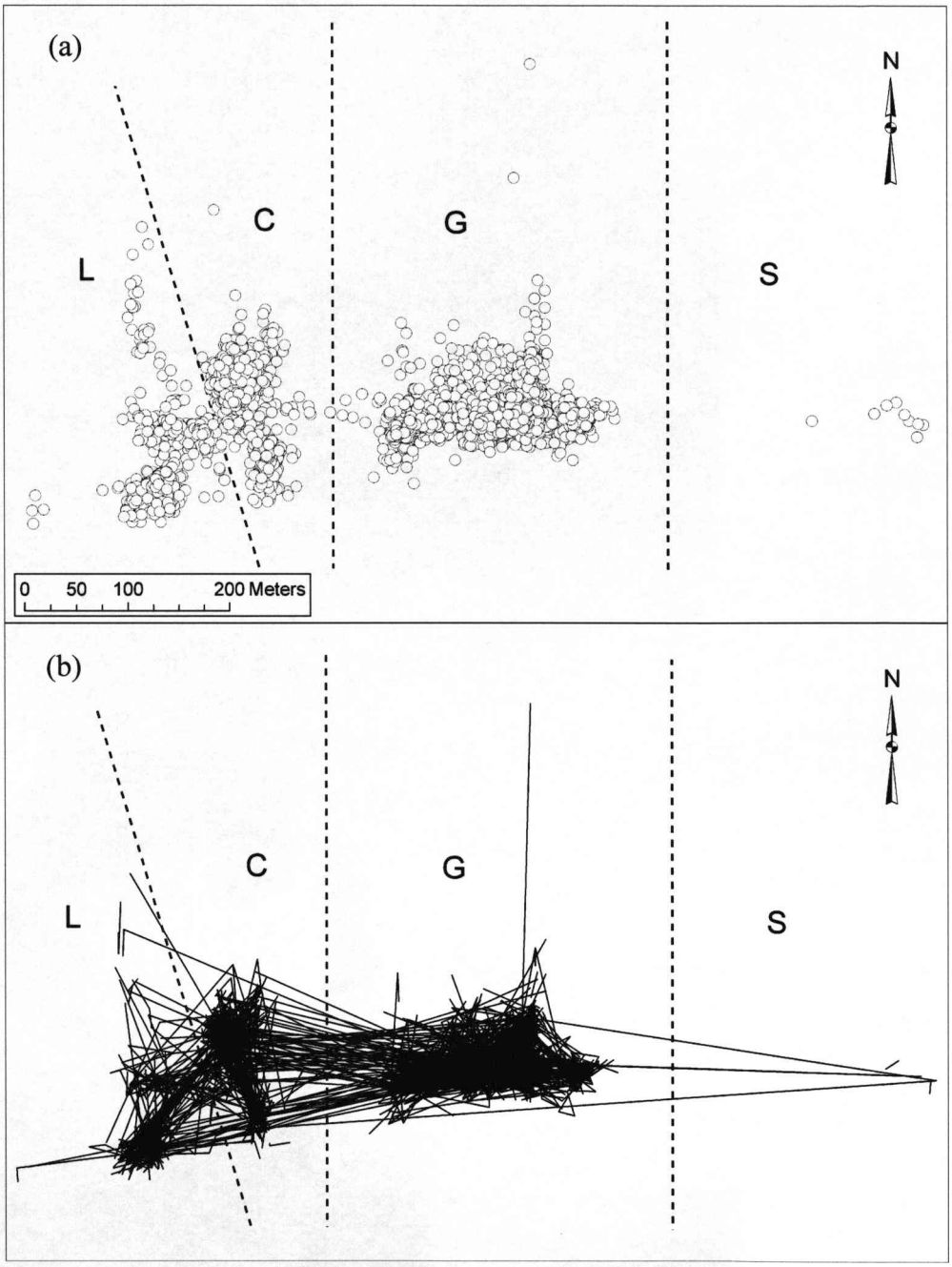


Figure 2.4. (a) Capture locations within Latchmoor (open circles) and (b) All movements within Latchmoor. Boundaries between sub-sites are indicated with dashed lines. L = Lay Gutter Valley, C = Concrete Henge, G = Gypsy Hollies and S = South Stream.

2.3 Results

2.3.1 Numbers marked and recaptured

In 2005, a total of 2052 *I. pumilio* was marked and released, comprising 1138 males and 914 females. Of those, 1215 individuals were recaptured, comprising 785 males and 430 females. There were 1126 complete records with no missing data and analyses were therefore restricted to these individuals unless otherwise stated. A breakdown of numbers marked and recaptured is presented in Table 2.2. The maximum number of times an individual was recaptured was 12 for a male and 11 for a female. The longest intervals between first and last capture were 31 and 30 days for males and females respectively.

In 2006, 252 individuals were marked and released, consisting of 162 males and 90 females. In total, 153 individuals were recaptured, comprising 98 males and 55 females. The maximum number of times an individual was recaptured was 14 for a male and 9 for a female. The longest time between first and last capture was 24 days for a female and 19 days for a male.

Table 2.2 Total numbers of adult *I. pumilio* marked and recaptured during each study. Recapture events refer to captures subsequent to marking.

	2005			2006		
	♂	♀	**Total**	♂	♀	**Total**
Marked individuals	1138	914	2052	162	90	252
Recaptured individuals	785	430	1215	95	55	150
Total recapture events	2237	872	3109	326	148	474
Mean lifespan (*T*) (days)	8.3	8.1	8.2	6.8	5.8	6.4
Mean time (*t*) (days)	1.9	2.5	2.0	1.6	1.9	1.6

There was no significant difference in the proportion of marked individuals recaptured in 2005 and 2006 (59.2% and 59.5% respectively; $\chi^2 = 8e^{-04}$, df = 1, $P > 0.05$). In 2005, males were significantly more likely to be recaptured than females (69.0% and 47.0% respectively; $\chi^2 = 100.1$, df = 1, $P < 0.001$). However, there was no significant difference between the sexes in 2006 (males 60.4% and females 61.1%; $\chi^2 = 0.0619$, df = 1, $P > 0.05$; Fig. 2.5; also see Chapter 4).

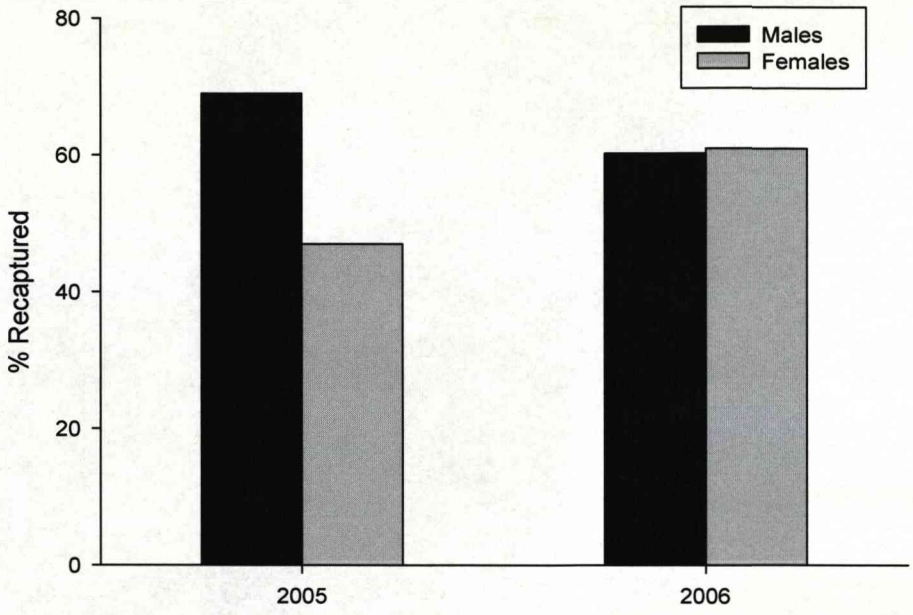


Figure 2.5. The percentage of marked individuals recaptured at least once during each study year. Males and females are shown separately.

2.3.2 Movement Patterns

In the 2005 study, the majority of individuals moved only a short distance in their lifetime, but a few travelled much further (Fig. 2.6). The overall median net lifetime movement (*N*) was 33m (geometric mean 31m), and 67.9% of individuals moved less than 50m (net) in their lifetime. However, maximum *N* was 391m and maximum gross lifetime movement (*G*) 1165m, both by males. Maximum female *N* was very similar to male at 389m, but maximum *G* much smaller at 575m.

The distribution of movement distances in 2006 was very similar to that in 2005 for small scale movements (Fig. 2.6). However, longer distance movements were absent from the 2006 study with a maximum *N* of 120m and maximum *G* of 317m, again both for males, with a similar female maximum *N* of 110m. The overall median *N* was 15m (geometric mean 16m) and 87.6% of individuals in this study moved less than 50m (net) in their lifetime. A full summary of the estimated movement parameters is presented in Table 2.3.

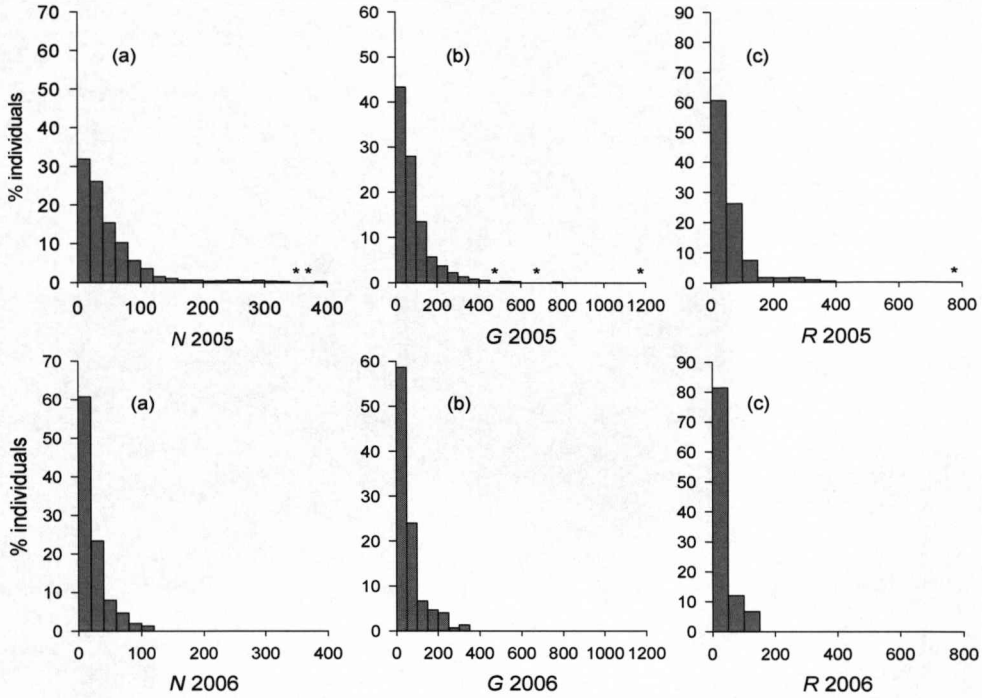


Figure 2.6. Percentage distribution of (a) net lifetime movements (*N*) in 20m distance categories, (b) gross lifetime movements (*G*) in 50m distance categories and (c) maximum range (*R*) during lifetime in 50m distance categories. N=1215 for 2005 and 150 for 2006. Bars marked '*' have a value between 0 and 0.1.

Table 2.3 Summary of all movement parameters estimated in both years. Means are geometric.

	2005			2006		
	♂	♀	**Total**	♂	♀	**Total**
Max N (*N*) (m)	391	389	391	120	110	120
Mean N (*N*) (m)	33	28	31	16	17	16
Max G (*G*) (m)	1165	575	1165	317	263	317
Mean G (*G*) (m)	64	43	56	39	29	35
Max range (*R*) (m)	751	389	751	120	148	148
Mean range (*R*) (m)	43	33	39	23	20	22
Max dist (*d*) (m)	747	389	747	120	148	148
Mean dist (*d*) (m)	21	21	21	12	11	12

In order to further examine the pattern of movements within the Latchmoor population, the site was divided into four sub-sites (Fig. 2.4). In all, 121 individuals (10% of all recaptured) transferred between sub-sites, of which 21 transferred twice and a further two individuals transferred three times. Captures within and movements between sub-sites are summarised in Table

2.4. Although there were areas of trees among the sub-sites, and Latchmoor Brook separates South Stream from the others, these cannot be considered significant barriers as movements were recorded to and from all sub-sites. The only two Latchmoor Brook sub-sites between which no movement was recorded were the two most distant from each other; Lay Gutter Valley and South Stream.

Table 2.4 Total numbers of adult *I. pumilio* marked and recaptured at each sub-site and movement events between sub-sites. Recapture values refer to captures subsequent to marking.

Site		Marked	Recaptured		Movements	
			Individuals	Events	From	To
Lay Gutter Valley	♂	91	107	274	21	47
	♀	65	36	75	8	9
	Total	156	143	349	29	56
Concrete Henge	♂	227	177	439	63	38
	♀	161	84	149	16	12
	Total	388	261	588	79	50
Gypsy Hollies	♂	815	560	1520	31	30
	♀	686	318	648	5	8
	Total	1501	878	2168	36	38
South Stream	♂	4	4	4	2	2
	♀	1	0	0	0	0
	Total	5	4	4	2	2
Beyond Latchmoor	♂	1	0	0	0	0
	♀	1	0	0	0	0
	Total	2	0	0	0	0
All sites	♂	1138	848	2237	117	117
	♀	914	438	872	29	29
	Total	2052	1286	3109	146	146

There was no significant effect of sub-site on the lifetime movement parameters estimated (1-way ANOVAs of $\log_{10}$ transformed distances: N, $F = 2.04$, df = 1211, $P > 0.05$; G, $F = 1.81$, df = 1211, $P > 0.05$; R, $F = 2.03$, df = 1211, $P > 0.05$). There was a significant difference between the first mature movement distances originating in different sub-sites (1-way ANOVA of $\log_{10}$ transformed distances: $F = 2.65$, df = 3104, $P < 0.05$). A Tukey HSD multiple comparisons test revealed that distances moved from

the South Stream sub-site were significantly different from all three other sites ($P < 0.05$ for each comparison), but that pair-wise comparisons between all other sites were non-significant. However, there were only 4 movement events from South Stream and two of those were transfers to another sub-site, so a much larger mean difference was likely given the distance between South Stream and other sub-sites.

To determine whether those individuals transferring between sub-sites were significantly larger than those remaining in the natal sub-site, an analysis of covariance (ANCOVA) was performed with wing length as the response. Individuals were categorised as either transferred or not transferred, which was added to the model as an explanatory variable. When analysing the size of mature damselflies it is necessary to remove the effect of emergence date, as size or mass at emergence has been shown to decrease as a season progresses (Banks & Thompson, 1985b; Anholt, 1990; Thompson, 1991). The estimated first day of maturity was therefore included as a covariate. Sex was also included as females are generally larger than males. Analysis of covariance revealed that transferring individuals did not differ significantly in wing length from their non-transferring counterparts and this variable was dropped from the model. Following stepwise deletion of terms, the significant predictors of wing length were sex (females larger) and first day of maturity (size declined through season). An interaction term indicated that female wing length declined slightly less than male (Table 2.5, Fig. 2.7).

Table 2.5 Significant predictors of *I. pumilio* wing length derived by ANCOVA simplified by backwards selection using *F*-tests on deletion. Starting model included a categorical variable for movement between sub-sites (see text) and all interactions. The *F*-value and associated P-value, df, R^2 and adjusted R^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates and standard errors (SE) are shown (* P<0.05, ** P<0.01, *** P<0.001).

Model summary	**Variable**	**Parameter estimates**	**SE**	***t***	**P**
$F = 353.5$	Sex	1.404	0.052	11.998	***
P = ***	1st day of maturity	-0.025	0.117	-9.514	***
df = 1122	Sex: 1st day maturity	0.010	0.003	2.210	*
$R^2 = 0.485$	Intercept	16.954	0.052	326.86	***
Adjusted $R^2 = 0.087$					

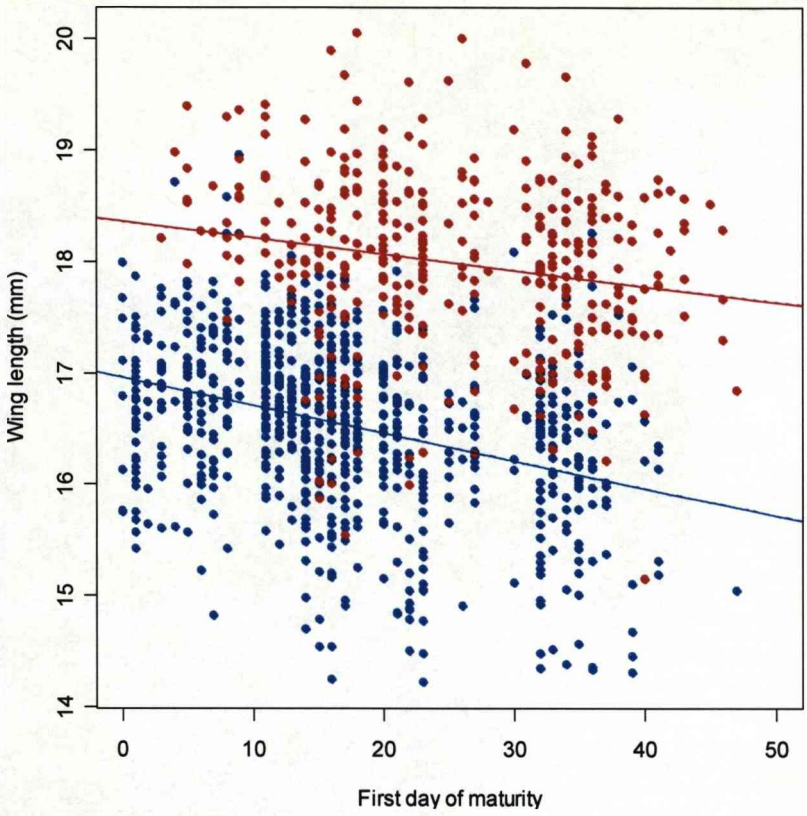


Figure 2.7. The effect of date in season (first day of maturity) and sex on body size (wing length) in *I. pumilio* (R^2 = 0.485, Adjusted R^2 = 0.087). Males are shown in blue and females in red.

Ischnura pumilio fits remarkably well into the relationship between size and dispersal probability reported in Conrad *et al.* (1999; Fig. 2.8). Species on the original graph were said to decrease in size generally from top to bottom and *I. pumilio* is plotted below them. The back transformed regression line is extremely similar to that for congeneric, *Ischnura elegans* which also exhibits weak flight but is known to disperse readily to new locations (Smallshire & Swash, 2004). This indicates that at least in good quality, maintained habitat, this species behaves as expected given its size based on the method of Conrad *et al.* (1999).

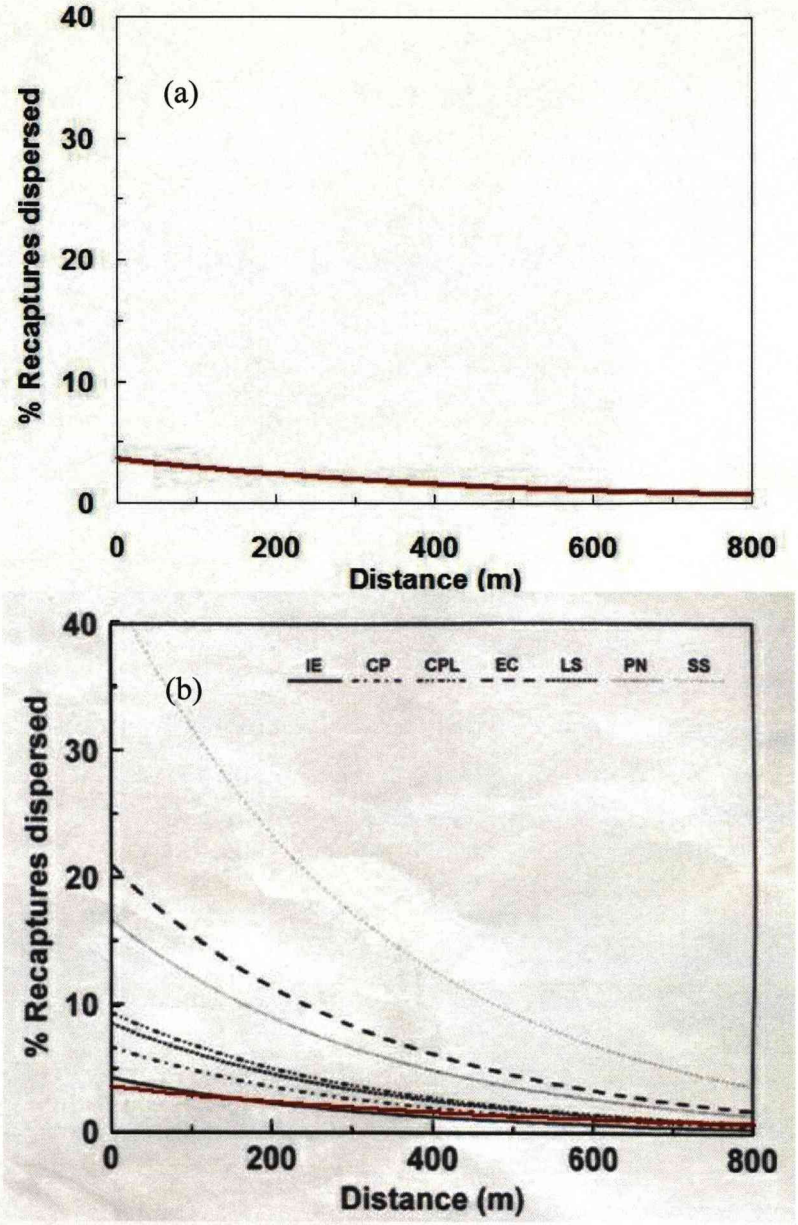


Figure 2.8. (a) Back transformed regression line derived from a linear regression of probability of dispersal ($\log_e$ transformed) in *I. pumilio* versus distance transferred. (b) The same regression line superimposed onto the graph produced by Conrad et al (1999). The original graph shows back-transformed regression lines, derived from an ANCOVA model testing for differences in mean dispersal probability among the main study species: IE – *Ischnura elegans*, CP – *Coenagrion puella*, CPL – *Coenagrion pulchellum*, EC – *Enallagma cyathigerum*, LS – *Lestes sponsa*, PN – *Pyrrhosoma nymphula*, SS – *Sympetrum striolatum*.

2.3.3 Movements beyond main sites

Despite regular searches of the surrounding area in 2005, only one male was found outside the main study site, approximately 1km away, and was not recorded again in the main site or otherwise. Similarly, a mature female was captured to the west of the main site approximately 800m from the nearest main-site capture, but also was not recaptured. As these distances are well within the range of lifetime movements recorded, and there was only a single individual in each case, they are likely to have originated from the main population.

Nine individuals were captured in the South Stream sub-site, some of which were either previously or subsequently recorded in the main site (Table 2.4). This sub-site was separated from the other three by greater distance, Latchmoor Brook and a considerably larger area of trees and unsuitable habitat. However, as transfers did occur and over distances well within the range observed in the rest of the site, these individuals were considered to be part of the main Latchmoor population.

In 2006, *I. pumilio* individuals were only recorded at Great Wheal Seton in significant numbers, and a further four individuals were captured at Rosewarne Mill. There were no observed movements between the sites though they are within the range of lifetime movements recorded in the 2005 study, at approximately 1km apart.

2.3.4 Factors affecting movement

Multiple regressions of the 2005 data showed a number of variables had a significant effect on movement distance in *I. pumilio*, and that these varied somewhat depending on the movement parameter considered. Factors significantly affecting distance moved (*d*) during the first mature movement are presented in Table 2.6. Time had a highly significant effect on *d*, with greater distances observed when the capture interval was longer. Slightly longer movements also occurred earlier in the season. The effect of density in starting locations was curved as indicated by the quadratic term. Smaller

movements occurred from areas of high density. The first principle component of the weather variables (PC1) was a significant predictor of distance moved. PC1 accounted for 38% of the variance in weather and was most related to temperature. At higher temperatures movement distances were slightly reduced.

Table 2.6 Significant predictors of distance moved during first mature movement (log_{10}) by *I. pumilio* derived by multiple regression. The *F*-value and associated P-value, df, R^2 and adjusted R^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates and standard errors (SE) are shown (* P<0.05, ** P<0.01, *** P<0.001).

Model summary	Variable	Parameter estimates	SE	*t*	P
$F = 22.52$	Time (log_{10})	0.28	0.033	8.486	***
P = ***	Date (log_{10})	-0.00459	0.001	-3.845	***
df = 1120	Weather PC1	-0.0304	0.011	-2.874	**
$R^2 = 0.09136$	Start density	-0.214	0.056	-3.809	***
Adjusted $R^2 = 0.0873$	(Start density)^2	0.0646	0.024	2.650	**
	Intercept	1.522	0.036	42.174	***

Net lifetime movement (*N*) was significantly increased in individuals with longer lifespans, and in those reaching maturity earlier in the season (Table 2.7). The effect of density was also significant and took a very similar form to the quadratic effect on *d*, with smaller movements occurring from high density locations (Fig. 2.9).

Table 2.7 Significant predictors of net lifetime movement (log_{10}) by *I. pumilio* derived by multiple regression. The *F*-value and associated P-value, df, R^2 and adjusted R^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates and standard errors are shown (* P<0.05, ** P<0.01, *** P<0.001).

Model summary	Variable	Parameter estimates	SE	*t*	P
$F = 33.75$	Lifespan (T)	0.0121	0.002	6.197	***
P = ***	1st day of maturity	-0.00437	0.001	-3.815	***
df = 1121	Start density	-0.316	0.060	-5.242	***
$R^2 = 0.1075$	(Start density)2	0.0864	0.026	3.284	**
Adjusted $R^2 = 0.1043$	Intercept	1.654	0.039	42.208	***

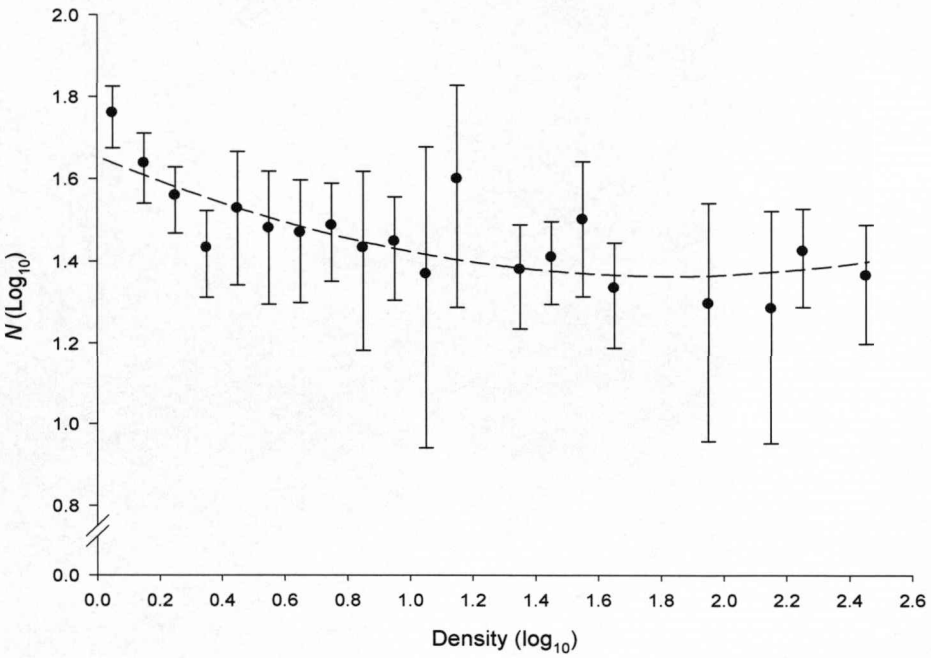


Figure 2.9. Effect of density on mean net lifetime movement (*N*). Vertical lines show 95% confidence intervals. Dotted line shows partial regression line derived from the multiple regression model (see text and Table 2.7). Density was calculated as the mean number of individuals marked or recaptured in each 10m × 10m section, and allocated to bins of 0.1 for calculation of mean *N*.

Lifespan was again highly significant in the analysis of gross lifetime movement (*G*), and included a quadratic term in this case. *G* increased with lifespan as before, but tailed off in those individuals living longest suggesting a potential trade-off between movement and survival, although this may merely reflect senescence (Table 2.8). Males moved significantly further than females, and the effect of density in starting locations was of the same quadratic form as in the preceding analyses. There was also a significant quadratic effect of wing length, whereby individuals with intermediate wing lengths travelled the furthest.

Factors significantly affecting range (*R*) were similar to those selected in the preceding analyses (Table 2.9). Males covered a significantly greater range than females. Range increased with lifespan but tailed off in individuals living longest and also decreased slightly in individuals maturing later in the season. Individuals with mites covered greater distances.

Table 2.8 Significant predictors of gross lifetime movement (log_{10}) by *I. pumilio* derived by multiple regression. The *F*-value and associated P-value, df, R^2 and adjusted R^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates and standard errors (SE) are shown (* P<0.05, ** P<0.01, *** P<0.001).

Model summary	Variable	Parameter estimates	SE	*t*	P
$F = 70.49$	Sex	-0.148	0.032	-4.627	***
P = ***	Wing length	0.599	0.253	2.365	*
df = 1117	(Wing length)^2	-0.0177	0.008	-2.356	*
$R^2 = 0.3313$	Lifespan (T)	0.0692	0.005	13.766	***
Adjusted $R^2 = 0.3271$	(Lifespan)^2	-0.00154	0.0002	-7.595	***
	Mites	0.0708	0.027	2.658	**
	Start density	-0.196	0.052	-3.775	***
	(Start density)^2	0.0722	0.022	3.208	**
	Intercept	-3.58	2.14	-1.676	ns

Table 2.9 Significant predictors of Range (log_{10}) by *I. pumilio* derived by multiple regression. The *F*-value and associated P-value, df, R^2 and adjusted R^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates and standard errors (SE) are shown (* P<0.05, ** P<0.01, *** P<0.001)

Model summary	Variable	Parameter estimates	SE	*t*	P
$F = 48.52$	Sex	-0.108	0.025	-4.377	***
P = ***	Lifespan (T)	0.0419	0.005	8.491	***
df = 1120	(Lifespan)^2	-0.00092	0.0002	-4.619	***
$R^2 = 0.178$	1st day of maturity	-0.00247	0.001	-2.395	*
Adjusted $R^2 = 0.1744$	Mites	0.0720	0.026	2.786	**
	Intercept	1.421	0.032	43.92	***

Overall these multiple regression analyses identified several variables with highly significant effects on the movement parameters considered. However, the effect sizes were generally small and the amount of variation explained low (R^2 values from 0.091 to 0.331). Males consistently moved further than females regardless of the movement parameter considered (Fig. 2.10), although sex was only significant in multiple regression analyses of gross lifetime movement and range.

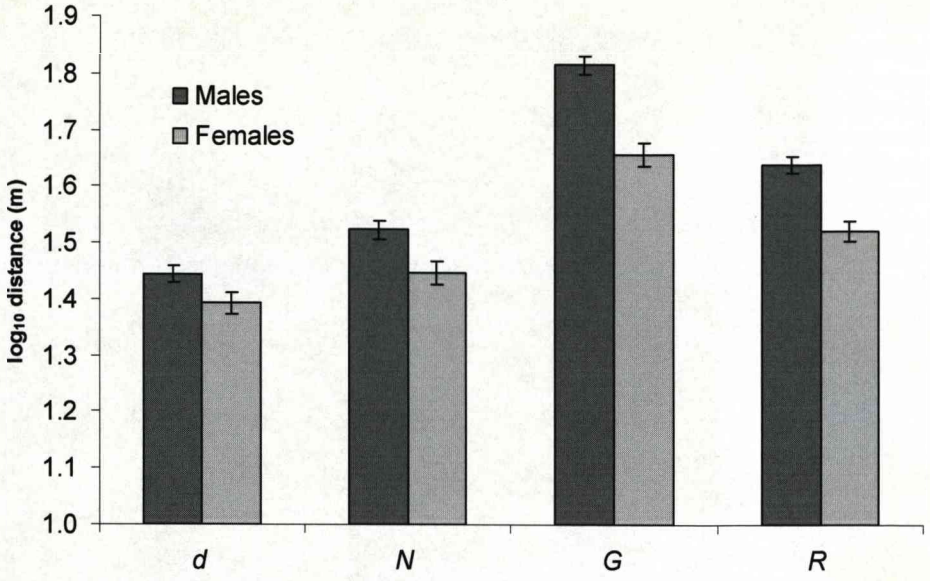


Figure 2.10. The effect of sex on mean distance moved for each of the movement parameters considered.

The effect of order of movement on individual movement distance was also examined (Fig. 2.11). Individuals travelled further on their first movement than any other, and distance declined with each subsequent move.

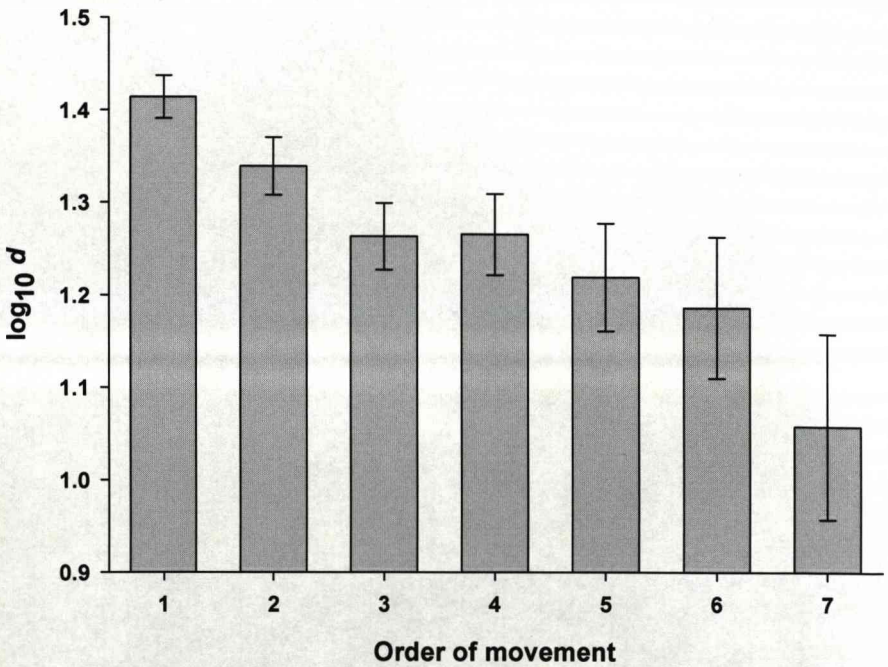


Figure 2.11. Effect of order of movement on $\log_{10}$ mean distance moved. Error bars represent 95% confidence intervals.

2.3.5 Direction of movements

The direction of first mature movements for each individual in 2005 is summarised in Fig. 2.12. There was a slight tendency for *I. pumilio* to move along the axis approximate to the east-north-east to west-south-west direction (circular mean of movement direction = 209°, where north - 0°), which was the direction of the main watercourse across the site, and the orientation of the distribution of suitable habitat. However, there were many other tributaries to this watercourse and the variety of movement directions may reflect this. The circular variance of the directional mean was 0.92. This suggests the mean was not a good representation of the set of input vectors (as the variance is close to one, its maximum) and that there was little directional bias to these movements (Gaile & Burt, 1980). However, if orientation is considered (the angle of movement without direction), the mean axis was 87° - 267° and the circular variance 0.32 (maximum 0.5 for a 180° distribution). This indicates that the orientation vectors were somewhat better represented by their mean and that movements along the angle of the main water body were more common.

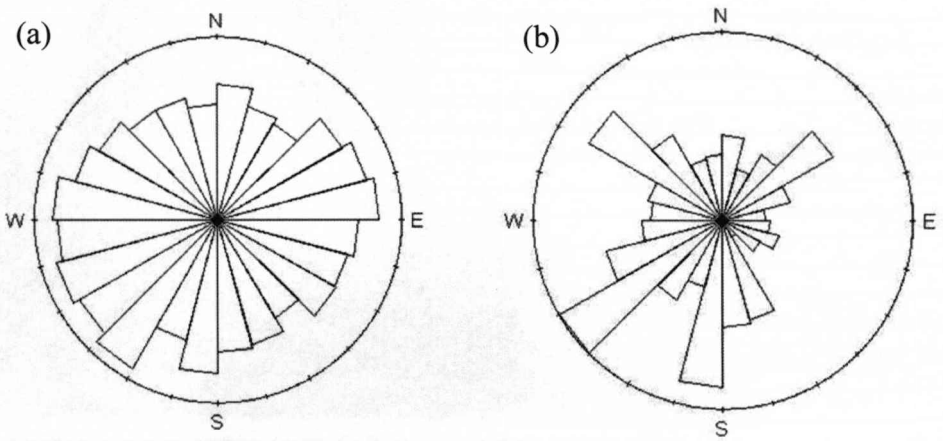


Figure 2.12. Rose diagrams showing the distributions of: (a) direction of first mature movements of *I. pumilio* at Latchmoor and (b) hourly wind direction measurements (from) between 10am and 7pm during the 2005 study period. Each bar represents 15°. Length of wedges represents proportion of directions.

To consider the effect of wind direction on direction of movement, the difference in angle between each first mature movement and the mean wind direction during that movement was calculated. As wind direction can vary considerably (Fig. 2.12; circular variance of wind directions between 10am

and 7 pm during study = 0.72), only those movements with an inter-catch interval of 1 or 2 days were used (n = 580). There was a small tendency towards movements approximately 50° different from the mean wind direction during that movement (Fig. 2.13). However, the circular mean of the difference between wind and movement direction was 85.7°, which is close to the median of possible differences (90°), and there was no strong trend in the distribution of differences (circular variance = 3.5, maximum 0.5 for 180° distribution). This suggests that *I. pumilio* adults are able to compensate for wind. This was true even at higher wind speeds, as there was no correlation between wind speed and the difference between movement and wind direction (Pearson correlation coefficient = 0.003, P = 0.94).

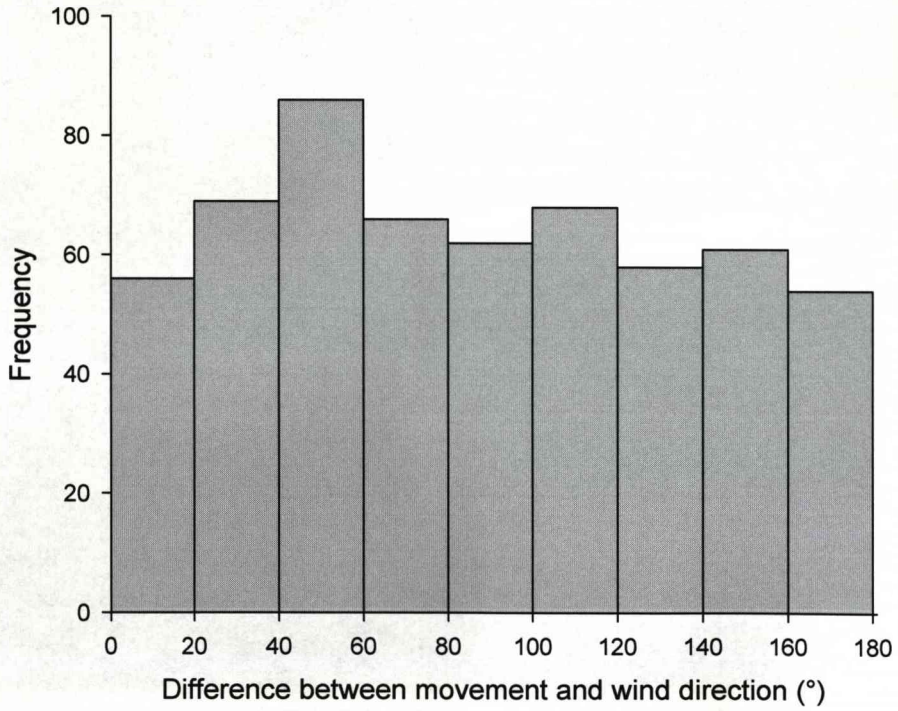


Figure 2.13. Frequency histogram of flight direction in relation to mean wind direction of first mature movements taking 1 or 2 days.

2.4 Discussion

2.4.1. Numbers recaptured

The percentage of individuals recaptured in this study (59.2% and 59.5% in 2005 and 2006 respectively) was considerably larger than that of similarly sized damselflies in previous MRR studies (e.g. Conrad *et al.*, 2002; Purse *et al.*, 2003; Rouquette & Thompson, 2007a). This may be because of limited dispersal in this species, resulting in more individuals remaining within the study area for recapture. It may also be partly due to the larger proportion of females recaptured in this study than in others (Garrison, 1978; Rouquette & Thompson, 2007a). A meta-analysis of MRR studies of damselflies found that males are generally more likely to be recaptured at least once than females (Beirinckx *et al.*, 2006). This was also true in 2005 in this study (to a lesser extent than most in the meta-analysis), but not in 2006 (Fig. 2.5). This may be because the 2006 study site was smaller with fewer individuals but the same search intensity was applied. When surveying large populations it is easy to overlook cryptic females as there are always conspicuous males to capture. However, when there are fewer individuals more time is spent searching and therefore finding females. Overall recapture rates were almost identical in the two study sites despite their considerable differences in area, spatial structure of suitable habitat and population size (see Chapter 4 for a detailed discussion). Over 100 observations of teneral individuals were made during this study, generally in areas close to water, which contrasts with the study of *I. pumilio* by Cordero Rivera & Andrés (1999) which failed to record a single teneral specimen.

2.4.2. Movement patterns

The distribution of net lifetime movements (*N*) estimated in 2005 was similar to that found for the similarly sized damselfly, *Coenagrion mercuriale* (Rouquette & Thompson, 2007a). However, in 2006 the distribution was similar only at short distances, and longer range movements were absent. Although both study areas encompassed several square

kilometres, the size of individual patches of suitable habitat in 2006 was much smaller. A linear relationship has been found between mean movement distance and size of study area in butterfly studies (Schneider, 2003). The same effect can be observed in this study: because there was no movement away from main sites, the size of the main site was the limiting factor rather than the size of the study area. Therefore, the difference in occurrence of long range movements in each study area is more likely to have been a feature of the distribution of habitat than of the populations themselves.

When gross lifetime movement (*G*) and range (*R*) are considered, the lifetime movement potential of *I. pumilio* can be better assessed. Maximum *G* recorded in 2005 was 1165m which is nearly three times greater than maximum *N*. Similarly, the maximum range covered by an individual in 2005 was 751m, almost double the maximum *N*. In 2006, maximum *G* and *R* were much reduced at 317m and 148m respectively, reflecting the smaller size of the main site. However, the value for *G* was again almost 3 times the maximum value for *N* (120m). By considering the sum of recorded movements (*G*), a better idea of the potential distance an individual can travel in its lifetime is obtained. Ultimately, this and individual movement distance (*d*) may be more useful than the frequently reported *N* when planning conservation management, as they allow decisions to be made about how close areas of breeding habitat and stepping-stone habitat patches must be.

I. pumilio is considered to be a weak flier - on a small scale at least - when compared to other odonates (Fraser, 1941; Fox, 1989; Smallshire & Swash, 2004) and this is supported by the findings of this study. Distance moved and dispersal probability have been found to increase with species size in a range of odonates (Conrad *et al.*, 1999; Angelibert & Giani, 2003) and in actively dispersing animals more generally (Jenkins *et al.*, 2007). Stettmer (1996) found that both *Calopteryx splendens* and *C. virgo* had a median movement distance (*d*) of about 50m and a maximum of 4km, compared to 21m and 750m respectively in this study of the much smaller *I. pumilio*. Of

the two *Calopteryx* species 42.5% of individuals moved less than 50m, compared with 80.0% and 92.6% in 2005 and 2006 respectively in this study. In 2005, movements over 200m were rare (1.9% of movements) and there was only one movement over 500m. In 2006, only 1.3% of movements were over 100m and no movements greater than 150m occurred. Of the similarly sized damselflies, *C. mercuriale* was found to move more than 500m in 1.3% of cases in one study (Rouquette & Thompson, 2007a) and in another study between 2% and 9% of males moved further than 200m, and between 4% and 14% of females (Purse *et al.*, 2003). Also within the Coenagrionidae, 73% of *Enallagma cyathigerum* moved less than 100m (Garrison, 1978).

There were no significant differences in movement parameters between the sub-sites in 2005. The high proportion of individuals transferring between sub-sites (12%) indicates that they were not so isolated as to cause genetic differentiation. Despite daily searches of all four sites in 2006 and regular searches of surrounding areas in both years, no movement away from the main site was observed in either year. As only four individuals were recorded at Rosewarne Mill, it is possible that they were colonisers from the stronghold population at Great Wheal Seton *c.*1km away, or that they were the offspring of a colonising gravid female in the previous year. As all four individuals were recorded within a week of each other, it seems improbable that they were all dispersers given the range of other sites where none was found.

However, of the entire area searched during 2006, Rosewarne Mill was the most similar to Great Wheal Seton in terms of habitat (see Chapter 3, Fig. 3.2), and if there were significant numbers of individuals dispersing this may have been the only site with the necessary cues for settlement. The highest maximum temperatures recorded during the 2006 study period were on the 7th (20.9°C) and 8th (25.6°C) June 2006. This coincided with the first capture recorded at Rosewarne Mill (7th June) and the other three captures followed within a week. As *I. pumilio* is reputed to disperse during the warmest weather (Fox, 1989; Cham, 1993), this peak in maximum daily

temperature may have prompted multiple dispersal events, four of which may have ended at Rosewarne Mill. Nevertheless, there is no firm evidence for dispersal events between sites in this study. Furthermore, no evidence of a metapopulation structure was found in 2006. This suggests that the long range dispersal reported previously in this species either occurs over larger distances than the scope of this (or any) MRR study, or that there is another explanation for the appearance of colonies far from other known populations.

As *I. pumilio* is often found in areas where there are no other odonates, and in habitat which appears unfavourable, it is likely that populations may have never been recorded due to their location (Dapling & Rocker, 1969; Fox, 1987). Along with an element of misidentification (Cotton, 1981), this has compounded the problem of recording the distribution of this species. Therefore, it is likely that there are many small transient colonies of *I. pumilio* which remain undiscovered and unrecorded for the duration of their existence (which may be as little as a year as the species is primarily univoltine; Cham, 1993; Cordero, 1994). These colonies may form stepping-stone populations, cutting the distance between known populations and facilitating movement across the landscape without the necessity for long range movement. This is further supported by the range of habitats in which the species can exist (see Chapter 3). Although natural habitat generally supports the more enduring populations, transient populations often occur in areas such as active quarry sites, bomb craters (Fox & Cham, 1994), motorway storm retention pools (Scher & Thièry, 2005) and even wheel ruts (Smallshire & Swash, 2004). As conditions are highly unstable in these types of habitat there will be constantly changing opportunities for colonisation, enabling progressive movements across the landscape.

In addition to the lack of evidence for long range dispersal from inter-site movements, there is also no evidence for the proposed long range dispersal mechanism of upward flight. Despite over 3000 man-hours spent in the field, over two flight seasons and in two geographical regions, not a single instance of upward flight as described previously (Fox, 1989; Cham, 1993)

was witnessed. During the study periods, weather conditions varied but were mostly clear, still and very hot: the conditions under which this behaviour was previously observed. On several occasions *I. pumilio* individuals were seen rising vertically into the air, but on all occasions they were observed alighting on a tree branch nearby, or moving horizontally to descend in an area of adjacent vegetation. It is possible that similar local movement events could be mistaken for movements to a much greater altitude on a bright day.

Following the 2005 study, it was considered possible that no extra-site dispersal events had been observed simply because the habitat was ideal, maintained and large. Therefore, there may have been no stimulus to disperse. It is likely that greater movement than that recorded is possible, as the early-successional habitat more typically occupied by populations of *I. pumilio* is prone to dry out or become overgrown, forcing populations to move on. It was partly for this reason that the study was repeated at the area chosen in 2006, as the habitat was more polluted, less maintained and comprised several smaller potential sites to which individuals could disperse. However, no long range dispersal movements were recorded.

2.4.3 Factors affecting movement

Distance moved during first mature movements was most affected by the length of time between captures. This has also been observed in *C. mercuriale* (Purse *et al.*, 2003; Rouquette & Thompson, 2007a), *Calopteryx splendens* (Schutte *et al.*, 1997) and *Enallagma cyathigerum* (Garrison, 1978). Similarly lifespan (time between first and last captures) had a highly significant effect on all of the lifetime movement parameters considered but effect sizes were small.

The distance moved by *I. pumilio* individuals during their first mature movement was inversely density dependent. Inverse density dependent movement was observed in a study of *C. mercuriale* (Rouquette & Thompson, 2007a), which attributed this unusual strategy to individuals

aggregating in areas of more suitable habitat. More commonly observed strategies are a tendency to spread out evenly within the available habitat in territorial species (Stettmer, 1996; Beukema, 2002), or classic density dependent dispersal, whereby the likelihood of movement increases in areas of high density. The latter is often said to be mediated by competitive effects on fitness or deteriorating environment due to crowding (Denno & Peterson, 1995; Matthysen, 2005). Inverse density dependent movement may be caused by conspecific attraction (Stamps, 1991), which can be explained as either benefits from aggregation such as decreased predation risk, or the use of conspecifics as a cue for resource or mate availability. It may be a response away from low density areas which may suffer Allee effects whereby aspects of fitness decline with population size (Allee, 1949; Kuussaari *et al.*, 1998; Stephens & Sutherland, 1999; Stephens *et al.*, 1999). Alternatively, the "social fence" hypothesis (Hestbeck, 1982; Matthysen, 2005) predicts that high densities reduce dispersal by inhibiting emigration through aggressive encounters.

There were several centres of high density within the Latchmoor site (Fig. 2.4) and the habitat at each was very similar (see Chapter 3, Fig. 3.2), with areas of increasingly different habitat having decreasing densities. For this reason it is difficult to disentangle the attractant effects of conspecific density and habitat, which are clearly related. A similar problem has been encountered in *C. mercuriale*, which has been shown to land preferentially on leaves where a male individual is already present (Martens, 2000) indicating an element of conspecific attraction; but has also been found to move greater distances away from certain habitat features, indicating an element of habitat selection (Rouquette & Thompson, 2005).

The presence of parasites on an individual during its life had a significant positive effect on the gross distance (G) it moved during its lifetime and the range (R) covered. A similar effect was observed in male *Coenagrion puella*, which were found to have an increased likelihood of dispersal when parasitised than when unparasitised, although no difference was found in females or *I. elegans* in the same study (Conrad *et al.*, 2002). This is

opposite to what might be expected, as mites are believed to considerably reduce activity in damselflies by draining body fluids and lowering condition (Smith, 1988; Reinhardt, 1996; Andres & Cordero, 1998). *Ceriagrion tenellum* exhibited decreased flight and mate searching efficiency when heavily parasitised (Andres & Cordero, 1998) and *Nehalennia speciosa* moved shorter distances (Reinhardt, 1996). Conrad *et al.* (2002) suggested that increased movement in parasitised individuals may be due to a higher energetic demand necessitating increased foraging activity. It may also represent a tendency to move away from areas with high mite density.

Males consistently moved further than females (Fig. 2.9), although this was only a significant predictor in the multiple regressions of *G* and *R*. Previous studies of damselflies have revealed contrasting evidence for sex differences in movement distance, but where present it is generally females that move further. Conrad *et al.* (2002) found that dispersing *I. elegans* females moved significantly further than males, but that there was no difference for *Coenagrion puella.* However, *C. puella* females moved significantly further than males in a study by Angelibert and Gianni (2003). Stettmer (1996) found no sex differences in *Calopteryx virgo* and *C. splendens*, again contradicting previous studies which found females to move further in both species (Waage, 1972). Two studies of *Coenagrion mercuriale* found no consistent differences between the sexes (Purse *et al.*, 2003; Rouquette & Thompson, 2007a). The increased values of gross lifetime movement and range for males may be reflective of their increased mate searching behaviour leading to increased overall movement, as opposed to more directed movement in females to and from areas for breeding and foraging.

The increased gross lifetime movement observed in individuals of intermediate size is an unexpected result as previous studies have found larger individuals to travel further. Conrad *et al.* (2002) found that dispersing male *I. elegans* had longer forewings than non-dispersing males, but there was no difference in females or in *C. puella*. Dispersing *Enallagma boreale* individuals were heavier at emergence than those

returning to their natal area to breed (Anholt, 1990), although the results of this manipulation experiment were contested by Thompson (1991) who found no difference in mass or wing length between dispersing and non-dispersing *C. puella*. The present study also found no difference in wing length between *I. pumilio* individuals transferring between sub-sites and those not transferring in either sex. The studies by Anholt (1990) and Thompson (1991) were of newly emerged individuals, kept overnight in either the field or laboratory and marked and released the following day. As stated by Thompson, this is highly likely to affect behaviour on release. In addition, the number of individuals recaptured in those studies was extremely low. By marking individuals after the teneral period, considering mature movements rather than maiden flights, and recapturing a significantly larger proportion of both transferring and non-transferring individuals, the present study should provide a more accurate analysis of movement in relation to size.

A negative effect of date in the season was observed on all movement parameters except gross lifetime movement. This was also observed in *C. mercuriale* by Rouquette & Thompson (2007a), who proposed two possible causes for a seasonal effect: phenotype and weather. Phenotype varies through the flight period and this can be observed as decreasing body size (wing length) at emergence as the season progresses (Anholt, 1990; Purse *et al.*, 2003; Rouquette & Thompson, 2007a; Fig. 2.7). Rouquette & Thompson suggested that a correlation between body size and movement would explain the observed pattern. However, body size was considered in this study and found to have a quadratic effect rather than a linear one. Similarly weather was also considered and found to have an effect only on first mature movement distance. The negative effect of date on movement distance was small, and may have been due to slightly increased mate searching behaviour early in the flight season when fewer individuals were present.

The first recorded movement was the longest and distance moved declined with each subsequent move in *I. pumilio*. This pattern has also been

observed in *C. mercuriale* (Rouquette & Thompson, 2007a). This may be a reflection of greater initial movements to find good quality habitat, followed by a reduction once found. In contrast the opposite pattern has been observed in *Sympetrum danae* (Michiels & Dhondt, 1991) and many butterflies (e.g. Warren, 1987; Bergman & Landin, 2002), where increased dispersal propensity or distance has been observed with increasing age or number of moves.

2.4.4 Direction of movements

There were no strong directional biases in the movement direction of *I. pumilio* although a tendency to move along main watercourses was suggested. This tendency has been observed in *C. mercuriale* (J.R. Rouquette & D.J. Thompson, unpub.), but this species inhabits a much more linear habitat than *I. pumilio*. *Sympetrum danae* showed a tendency to move away from areas of trees, and males oriented more southwards than females (Michiels & Dhondt, 1991). An ability to compensate for wind direction even at the highest wind speeds measured was demonstrated, and served to confirm field observations of relatively strong flight against gusts of wind. A study of *Pantala flavescens* (Feng *et al.*, 2006) found an ability to compensate for headwind drift during large scale movements at high altitude. However, a radio-telemetry study of similar movements in *Anax junius* found that this species did not compensate strongly for wind direction (Wikelski *et al.*, 2006). As *I. pumilio* is considerably smaller than either of these examples, and has previously been thought of as a weak flier, it is somewhat surprising that movement direction is not more strongly affected by wind.

Much of what was previously thought about the movement characteristics of *I. pumilio* has been thrown into doubt by the findings of this study. No evidence for long range movement has been found, nor was the proposed mechanism for these events, upward flight, observed at all during the study. Dragonflies are notoriously difficult to track due to their accomplished flight and few studies have managed to produce quantitative records of

movements other than short range (Corbet, 1999). Those that have succeeded used equipment such as radio transmitters (Wikelski *et al.*, 2006) which is currently too heavy to be fitted to *I. pumilio* and allow free movement, or radar equipment (Feng *et al.*, 2006) which was beyond the scope of this study. On the other hand the weak flight observed when close to the ground has been shown to have the ability to compensate for wind direction. This contradicts previous observations which have suggested even weak gusts of wind can cause considerable displacement (Dapling & Rocker, 1969).

2.4.5 Dispersal probability in relation to size

Using the method outlined in Conrad *et al.* (1999), *I. pumilio* appears to fit well into the pattern of decreasing dispersal probability (between sub-sites) in smaller odonates (Fig. 2.8). However, this method does not consider those individuals which did not move between (sub-) sites. Furthermore, the species are not strictly in order of size from top to bottom as suggested by Conrad *et al.* (1999). As such the relationship shows only that mean dispersal probability decreases with distance and that species vary in their mean dispersal probability. Dispersal declined with distance at a similar rate for each species on the original exponential scale.

A more robust method for considering movement potential relative to size uses 1% movement distances (distance moved by 1% of individuals) for all individuals, rather than distances moved only by those transferring between areas. *Ischnura pumilio*'s observed 1% movement distance (638m in Latchmoor 2005) is greater than that predicted given its size while most other odonates fit the relationship well (C. Hassall & D.J. Thompson, unpub.). Given the difficulties in estimating the tail of the dispersal kernel, which is clearly important in determining a species' persistence in a fragmented landscape, the use of complete data sets may be more informative. In any case, the study area should be sufficiently large to include long range movements (Schneider, 2003).

2.4.6 Summary

The limited movement shown by *I. pumilio* in this study has implications for its conservation. Management should involve creation of new habitat in areas close to existing populations, within the range of movement distances observed in this study, and maintenance of existing habitat in a suitable state (see Chapter 3).

Southwood (1962) proposed that insects should evolve to have a level of dispersal capability related to the permanence of their habitat. *Ischnura pumilio* inhabits inherently ephemeral, early-successional habitats, but there was no evidence of a high dispersal capability in this study. However, the two main sites considered are unusually permanent with respect to the range of habitats utilised by the species: the first maintained by grazing and the second by residual pollution from the tin mining industry. These populations may have lost the genes for dispersal, due to the increased costs associated with the uncertain outcome of dispersal compared with remaining at the natal site (Dieckmann *et al.*, 1999; Watts *et al.*, 2004). More generally, limited dispersal capability may be a result of past landscape stability mediated by centuries of traditional land management, rather than the present situation of rapid change and loss of wetlands.

This study is the first to quantitatively assess the movement potential of *I. pumilio* and has provided reliable estimates of the species local (trivial) movement ability. Corbet (1999) identified four types of non-trivial flight in odonates: the maiden flight, commuting, seasonal refuge and migration. Of these, the present study has only considered commuting flights between breeding and roosting sites, which were included by surveying the entire habitat including areas away from water. Seasonal refuge is not applicable to *I. pumilio* as adult life does not span seasons. Maiden flights cannot be reliably quantified by MRR techniques without the risk of damage to delicate, teneral individuals or potential effect on behaviour (Thompson, 1991). Migration can occur within and above the insect flight boundary layer (the few metres of air above the earth's surface within which wind speed permits active orientation: Corbet, 1999), and it is those movements above that boundary which may also be missing from this study. Odonates

transported by wind can cover great distances in random directions from the source and alight in areas far from known populations (Corbet, 1999). The quantification of these movements was unfortunately beyond the scope of this study.

The direct measurements of dispersal presented here and lack of evidence for metapopulation structure suggest that populations are significantly isolated from each other. Forthcoming genetic analysis of these populations will provide further evidence of the degree of isolation between adjacent colonies and the effect of distance, although previous comparisons have found strong correlation between demographic and genetic measures of dispersal in a damselfly (Watts *et al.*, 2006) and a butterfly (Vandewoestijne & Baguette, 2004). Creation of patches of suitable habitat between existing populations may serve to connect isolated sites and allow a degree of genetic mixing.

The finding that movement in this species is inversely density dependent also has important conservation implications if density itself is the cue for emigration. Small isolated populations will be more prone to extinction if individuals attempt to emigrate due to low density (Rouquette & Thompson, 2007a). This further enhances the importance of landscape connectivity, as new colonisers will be required to resurrect extinct populations if their persistence is to be ensured.

Chapter 3: Habitat requirements of adult *Ischnura pumilio*

3.1. Introduction

The abundance and status of *Ischnura pumilio* populations in the UK is not well known, but the species is thought to have been in decline in recent years and certainly has a sparse, localised distribution (see Chapter 1). Like all odonates it is restricted to wetland habitats for larval development, which have declined by 50% in the UK since 1945 (Nature Conservancy Council, 1984). In order to avoid further decline of this species, an understanding of the important features of its habitat must be developed and sites preserved in a suitable condition for its persistence. Despite its supposed specialised habitat requirements, *I. pumilio* is found in a variety of habitat types in the UK, at a variety of altitudes and levels of disturbance and pollution (Cotton, 1981; Fox, 1989; Fox & Cham, 1994). However, the unifying features of its habitat based on previous observational data are shallow water, with a very slow flow rate and a limited amount of emergent vegetation (Fox, 1989; Cham, 1991).

Ischnura pumilio is restricted to southern and western sites in the UK, and does not occur where the February minimum temperature isotherm is below 2.2°C (Chelmick, 1980; Fox, 1990). At this northern margin of its global range, the species may have narrower or different niches than further south (Thomas, 1994). However, changing climatic conditions are expected to cause species' ranges to extend towards the poles and higher altitudes. This has been documented across taxa (Hickling *et al.*, 2006) and in the Odonata specifically (Hickling *et al.*, 2005; Hassall & Thompson, 2008). Such range expansion may be accompanied by changes in the availability of habitat areas. An understanding of what constitutes an organism's habitat is therefore crucial, if successful conservation management is to continue through changing climatic and environmental conditions.

Surveys of odonates and their habitat are commonly conducted during warm, sunny weather, when adults are active and mating near to water. Structural components of the habitat surrounding areas of water are often ignored (but see Stoks, 2001a), despite their importance for roosting and feeding (Foster & Soluk, 2006; Rouquette & Thompson, 2007b). However, essential to conserving a species is identifying the extent of the habitat and the resources utilised within it. Without clearly defining what constitutes habitat as distinguished from the surrounding matrix, studies of dispersal between habitats become problematic. Habitat use may change with daily variation in weather (Dennis & Sparks, 2006). Hedges, shrubs and other low level vegetation are utilised at night for roosting (Rouquette & Thompson, 2007b) and by females between bouts of mating and oviposition (Banks & Thompson, 1987; Anholt, 1992; Stoks, 2001a; Stoks, 2001b). They may also be used during cool, cloudy and windy conditions for shelter and due to insufficient warmth for flight to water. As such, the focus on areas close to water does not encompass the full range of habitat utilised by damselfly species.

Odonate species composition at a site will depend on the particular characteristics of that site. Features such as water depth, flow rate and pH (Smallshire & Swash, 2004) will favour certain species and deter others. These environmental features will also influence the vegetation community and structure at a site. As *I. pumilio* is often over-looked due to its small size and ephemeral habitat use (Dapling & Rocker, 1969; Cotton, 1981; Fox, 1987) a combination of odonate and plant species composition, along with abiotic measurements, may provide a useful indicator of the likelihood of *I. pumilio*'s presence at a site. Multivariate methods allow the relationship between a multivariate response (odonate composition) and multivariate predictors (vegetation and environmental variables) to be explored. These methods were employed to determine the association between *I. pumilio* and other odonates, and the variables which influence these associations.

This Chapter reports the results of a detailed assessment of the habitat requirements of *I. pumilio* and its use of different areas within that habitat. Specifically the following questions are addressed:

- How are environmental variables related to odonate species composition?
- Which odonate species are associated with *I. pumilio*?
- What are the significant predictors of *I. pumilio* abundance at a site?
- How is use of different areas within a habitat influenced by sex and weather?

3.2. Methods

3.2.1. Study sites

In total, 31 sites across Cornwall, Devon and Hampshire were surveyed during summer 2006 (see Table 3.1). Sites were chosen at random from a list of those with a recorded sighting of *I. pumilio* during the last 10 years, provided by the respective vice-county recorders for the British Dragonfly Society (Smallshire, 1996; Jones, 2006; D. Jenkins pers. com.). Also included for comparison were two sites recommended by the Cornwall vice-county recorder as appearing ideal but having no past records (Wimal Ford and Newlyn East Downs). Sites varied from natural, undisturbed areas (e.g. Latchmoor, New Forest) to previously industrialised sites (e.g. Great Wheal Seton) and other highly disturbed or man made sites (e.g. Smallhanger Waste, Devon and Sand Cottages, Cornwall). Several sites were divided into sub-sites because they were either very large (Latchmoor) or naturally formed two distinct areas in terms of habitat type and *I. pumilio* presence (Rosewarne and Chapel Porth). One site (Menadue) was omitted from analyses as no water was present at the site resulting in missing data values.

A suite of environmental variables was recorded at the site level and within quadrats: a summary of these is presented in Table 3.2. Quadrats were positioned along transects emanating from randomly selected points within the area of water at each site. Six transects were defined at each site, each consisting of 5 quadrats placed at 0, 2, 5, 10 and 20m from the start point within water. An example of the sampling design is shown in Appendix 1. National Vegetation Classification (NVC) classes for each site were assigned using Tablefit 1.0 (Hill, 1996). Where two vegetation types had equal goodness of fit the type with better compositional satisfaction was selected.

Table 3.1. Table of site names and codes, grid references (centre of site), NVC community codes, NVC goodness of fit values and *I. pumilio* abundance. Letters in parentheses following Latchmoor refer to sub-sites defined in Chapter 2, Fig. 2.4. Site codes commencing "A" are in Cornwall, "B" in Devon and "C" in Hampshire. NVC community definitions are: A24, *Juncus bulbosus* community; H9, *Calluna vulgaris-Deschampsia flexuosa* heath; M6, *Carex echinata-Sphagnum recurvum/auriculatum* mire; M15, *Scirpus cespitosus-Erica tetralix* wet heath; M23, *Juncus effusus/acutiflorus-Galium palustre* rush-pasture; M25, *Molinia caerulea-Potentilla erecta* mire; M29, *Hypericum elodes-Potamogeton polygonifolius* soakway; MC8, *Festuca rubra-Armeria maritima* maritime grassland; MC9, *Festuca rubra-Holcus lanatus* maritime grassland; S10, *Equisetum fluviatile* swamp; S11, *Carex vesicaria* swamp; S25, *Phragmites australis-Eupatorium cannabinum* tall-herb fen; SD17, *Potentilla anserina-Carex nigra* dune-slack community; W1, *Salix cinerea-Galium palustre* woodland; W4, *Betula pubescens-Molinia caerulea* woodland; W25, *Pteridium aquilinum-Rubus fruticosus* underscrub. Lower case letters refer to sub-communities which are not defined here due to low GOF values.

Site	Code	GPS easting	GPS northing	NVC	GOF	N° *I. pumilio*
Great Wheal Seton	Aa	165511	41795	S11c	18	26
Roscroggan	Ab	165186	42055	W1	21	0
Rosewarne 1	Ac	164469	41739	S10	16	0
Rosewarne 2	Ad	164477	41815	S25	12	3
Bell Lake Marsh	Ae	162163	41884	S10	12	0
Peter's Point	Af	157706	41045	MC9c	26	0
Chapel Porth 1	Ag	169991	49349	S10	14	0
Chapel Porth 2	Ah	169837	49428	MC8	19	11
Wimal Ford	Ai	221241	73469	M15	49	0
Sand Cottages	Aj	178934	39707	A24	13	1
Penwithick	Ak	202049	56274	M23	16	7
Stepper Point	Al	191494	78363	SD17c	10	53
Menadue	Am	202791	59385	M29	24	0
Tolgus tin	An	168830	44783	W25b	15	0
Carbis Moor	Ao	202797	55882	W4	7	2
North Tresamble	Ap	174709	40468	A24	18	0
Newlyn East Downs	Aq	183618	54353	H9e	17	0
Prewley Moor	Br	254226	90996	M25b	15	4
Sheepstor	Bs	257837	68200	M25a	42	0
Cadover Bridge	Bt	255615	64824	M23	29	51
Blackabrook	Bu	256936	63984	M6a	27	13
Smallhanger Waste	Bv	257623	59487	M25a	31	29
Whitchurch Down	Bw	250816	74189	M25	33	0
Wigford Down	Bx	253725	64953	M25a	45	0
Lydford Railway	By	250057	82595	M25	40	7
Walla Brook	Bz	267066	77138	M6a	33	6
Latchmoor 1 (L)	Ca	419402	112778	M25	20	58
Latchmoor 2 (C)	Cb	419147	112759	M29	23	23
Latchmoor 3 (G)	Cc	419064	112705	M25a	29	19
Shipton Bottom	Cd	436269	99603	M25a	26	3
Millersford Bottom	Ce	418650	116705	M25a	50	0

Table 3.2. Potential predictors of odonate species composition and *I. pumilio* abundance, transformations applied (in parentheses) and number of selections by step procedure in CCA analysis. Letters in parentheses indicate plant groups which were combined due to collinearity in the GLM of *I. pumilio* abundance, each letter represents a different set. Variable names in parentheses are used in Fig. 3.2.

Variable	Description	Selections
Easting	British national grid location (Log_{10})	12
Northing	British national grid location (Log_{10})	16
Altitude	As recorded by handheld GPS (m) (Log_{10})	0
Area	Area of potential habitat (m^2)estimated from map (Log_{10})	
Bankangle (bank)	Clinometer reading between transect points 0 & 10 (m) (None)	6
Disturbance	Human disturbance: 0, relatively untouched; 1, partially disturbed, some management, pathways, nearby roads; 2, industrial, heavily used sites (None)	8
Grazing	0, none; 1, light/occasional; 2, heavy. (None)	11
Shade	% shade over quadrat (Arcsine)	20
Type	Stream/flush/bog (Arcsine)	0
Mudcover	% cover of bare mud (Arcsine)	0
Muddepth (mud)	Depth of mud (cm) in centre of quadrat (Log_{10})	2
Watercover	% cover of water (Arcsine)	0
Waterdepth (water)	Depth of water (cm) in centre of quadrat (Log_{10})	20
Substrate	Silt/silt & gravel/gravel	8
Turbidity	Proportion of standard grayscale visible through standard tube of water from quadrat (Arcsine)	0
Conductivity (cond)	µSiemens measured in quadrat	3
Flowrate	Measured in m s^{-1} using 1cm^2 of polystyrene (Arcsine)	2
Maxheight	Maximum height of vegetation within quadrat (cm) (None)	7
Structure zone 0 (z00)	Structural complexity index at transect point 0m (None)	3
Structure zone 2 (z02)	Structural complexity index at transect point 2m (None)	0
Structure zone 5 (z05)	Structural complexity index at transect point 5m (None)	0
Structure zone 10 (z10)	Structural complexity index at transect point 10m (None)	3
Structure zone 20 (z20)	Structural complexity index at transect point 20m (None)	14
Spcount (sp)	Number of odonate species observed at site (Log_{10})	7
ACG	Aquatic sedge/grass (% cover) (Arcsine)	0
AFO	Aquatic forb (% cover) (Arcsine)	0
AH	Aquatic horsetail (% cover) (Arcsine) (a)	2
MC	Marginal sedge (% cover) (Arcsine) (b)	3
MFO	Marginal forb (% cover) (Arcsine)	0
MG	Marginal grass (% cover) (Arcsine) (b)	16
MM	Marginal moss (% cover) (Arcsine)	8
MR	Marginal Rush (% cover) (Arcsine)	0
TC	Terrestrial sedge (% cover) (Arcsine) (c)	0
TD	Terrestrial dwarf shrub (% cover) (Arcsine)	14
TFE	Terrestrial fern (% cover) (Arcsine) (a)	1
TFO	Terrestrial forb (% cover) (Arcsine)	2
TG	Terrestrial grass (% cover) (Arcsine) (c)	5
TR	Terrestrial rush (% cover) (Arcsine)	2
TS	Terrestrial shrub (% cover) (Arcsine) (b)	0
TT	Terrestrial tree (% cover) (Arcsine)	0

Plant species were grouped to reduce the number of variables to be considered. Table 3.2 contains a full list of groups which were based on functional group and habitat (aquatic, marginal or terrestrial). Means of explanatory variables measured at the transect or quadrat level were calculated for each site. Geometric means of percentages were calculated (Burns, 1929); zeroes in the data were converted to a value of $1x10^{-6}$ and means estimated at this value converted back to zero (De Muth, 2006). Coverage of each plant group at a site was calculated as the sum of the geometric mean coverage of all species in the group.

Plant structural complexity was measured by placing a marked pole in the centre of each quadrat, and recording the number of contacts by plants in each of the vertical intervals 0-10, 10-30, 30-70, 70-150 and over 150 cm (Lawton & Woodroffe, 1991). These measurements were then reduced to a compound measure of structural complexity for use in the following analyses by using their first principal component. Structural complexity was considered separately at each distance from water (hereafter referred to as zones) sampled along transects. *Ischnura pumilio* and other odonates are thought to require a limited amount of emergent vegetation for oviposition, without the water becoming overgrown, however increased structural complexity was expected to be preferred as distance from water increased as a source of shelter and food.

One site was surveyed per day by two people. No surveys were conducted during rain or strong winds. All *I. pumilio* individuals sighted between 11am and 4pm whilst conducting the site survey were captured and marked to ensure they were not recounted. Half an hour was also spent exclusively searching for *I. pumilio* during that time. This enabled a comparable count of *I. pumilio* at each site to be obtained. Numbers of all other odonates observed throughout the day were estimated using RA83 codes. The RA83 recording system (formerly RA70) is that used on standard odonate recording cards available from the Biological Records Centre (http://www.brc.ac.uk/record_cards.asp). The system provides codes for abundance and behaviour categories and is widely used for recording

odonates in the UK. The median of the abundance range for each RA83 code was then used in the following analyses.

3.2.2. Odonate habitat associations

In order to examine the associations between odonate species composition at sites and the abiotic factors and plant species (hereafter referred to as environmental variables), multivariate (ordination) analyses were employed. One of the main goals of ordination methods is to produce a two dimensional plot which reveals patterns and relationships between objects (here sites and species). The ordination plot represents the differences in species composition between sites by the plotted distances between them. Co-occurrence of species is also represented by a smaller plotted distance. Ordination axes represent environmental gradients to which the species are responding, and may be related to measured environmental variables by adding them to the model as explanatory variables and subsequently to the plot.

The validity of multivariate methods was checked initially by conducting detrended correspondence analysis (DCA; Hill & Gauch Jr., 1980) and non-metric multi-dimensional scaling (nMDS; Manly, 2004) of the odonate composition data from each site. Both methods are considered robust, so if they produce similar ordinations multivariate methods are likely to produce stable results, (Fig. 3.1). Correspondence analysis (CA) finds ordination axes (environmental gradients) which maximise the correspondence between sites and species. It then uses reciprocal averaging to find site and species values along those axes. DCA is a variant of CA in which ordination axes are rescaled in order to remove curvature in species responses which is considered to be an artefact of the ordination method. nMDS is fundamentally different in design from other ordination methods and is considered to be the most generally effective for ecological community data (Legendre & Legendre, 1998; McCune & Grace, 2002). It uses observed dissimilarities between objects (sites and species) to map the objects in two

dimensions, minimising the stress on dissimilarities from the original multi-dimensional solution.

Table 3.3. Odonate species, codes used in ordination plots, frequency of occurrence across the 31 sites surveyed and frequency of co-occurrence with *I. pumilio.*

Species	Code	All sites	*I. pumilio* sites
Calopteryx virgo	C.vi	8	3
Ceriagrion tenellum	C.te	13	10
Coenagrion mercuriale	C.me	6	5
Coenagrion puella	C.pu	21	13
Enallagma cyathigerum	E.cy	5	5
Ischnura elegans	I.el	20	13
Ischnura pumilio	I.pu	17	n/a
Lestes sponsa	L.sp	5	5
Pyrrhosoma nymphula	P.ny	22	12
Aeshna juncea	A.ju	1	7
Anax imperator	A.im	12	0
Cordulegaster boltonii	C.bo	9	5
Libellula depressa	L.de	9	4
Libellula quadrimaculata	L.qu	7	3
Orthetrum cancellatum	O.ca	2	1
Orthetrum coerulescens	O.co	23	14
Sympetrum danae	S.da	1	1
Sympetrum striolatum	S.st	2	1

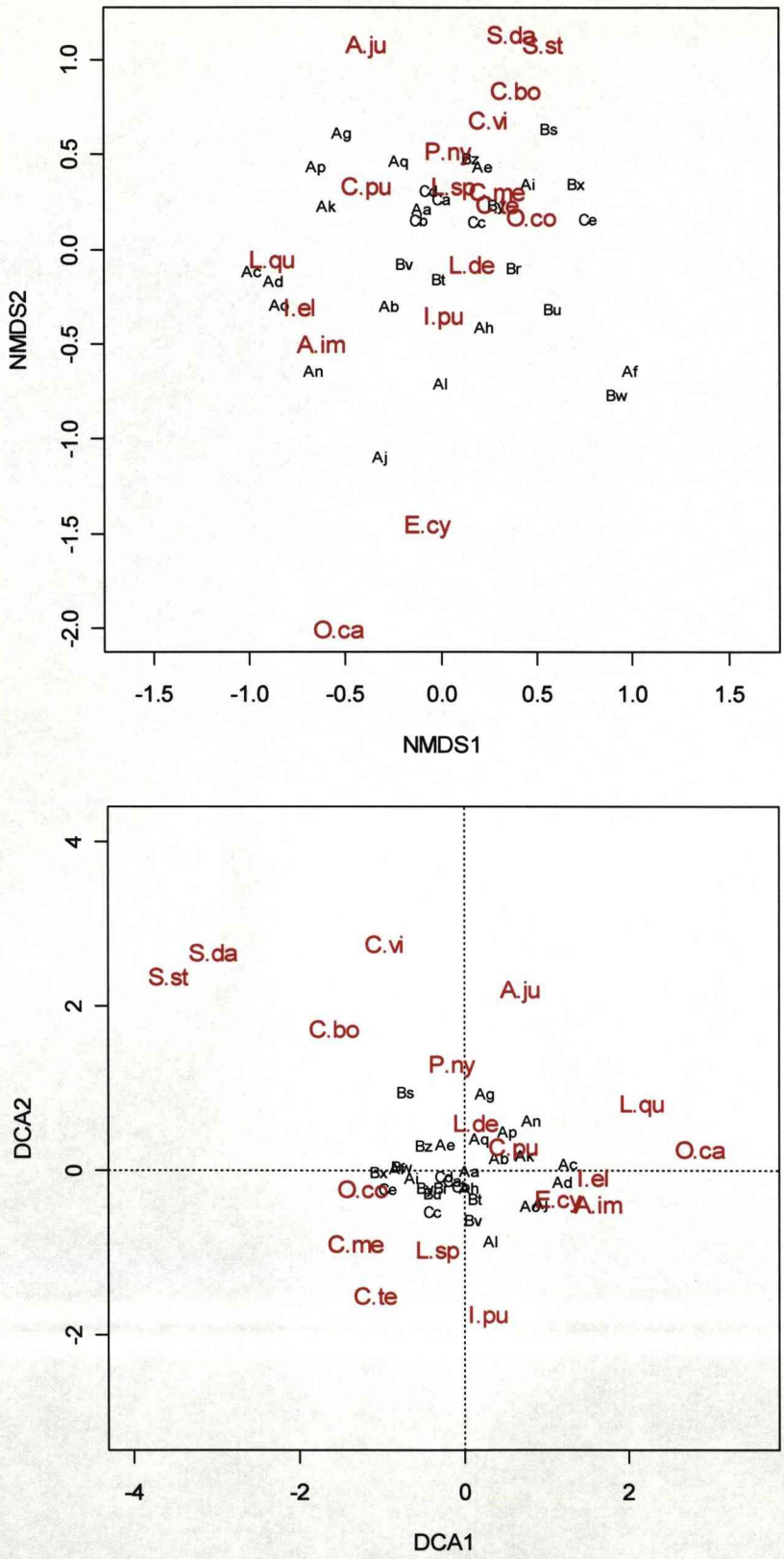


Figure 3.1. Non-metric multidimensional scaling (NMDS; top) and detrended correspondence analysis (DCA; bottom) of odonate count data from each site showing similar groupings of species. Species occurring in less than 20% of sites were down-weighted. Site codes in black are explained in Table 3.1 and species codes in red in Table 3.3.

An unconstrained CA of odonate species produced a scatter of 6 ordination score units along axis 1 and 5 ordination score units along axis 2. *Enallagma cyathigerum* and *Orthetrum cancellatum* were outliers which strongly influenced this CA solution, and down-weighting species present in less than 20% of sites lowered the variation in scatter of species points. Constrained correspondence analysis (CCA) was then performed on the same data in order to examine the variation in species data due to measured environmental variables. CCA considers only the community structure that is related to the measured environmental variables, and allows them to be subjected to model selection in a similar way to multiple regression.

Model selection followed several stages due to the large number of potential explanatory variables. Initially, the set of environmental variables was split into three random subsets and this was repeated 10 times. Each subset was used to obtain a maximal model. The variance inflation factors (VIFs) were checked for each maximal model, and those variables with VIFs greater than 10 (indicating correlation with other variables) were removed before model selection (Oksanen, 2008). These maximal models were then subjected to backwards and forwards selection based on Akaike's Information Criterion (AIC). The full set of variables was therefore fitted to the odonate data 20 times (10 forwards and 10 backwards) in random subsets. The variables selected in these analyses were then combined for further model selection following the same procedure of forwards and backwards selection using AIC.

Constrained ordination methods do not have a log-likelihood and therefore do not have a true deviance from which to derive AIC. Instead, AIC is the goodness of fit derived from the residual inertia (variance) penalized by the degrees of freedom. Although these methods are implemented in Vegan 1.13-0 and are used by the backward and forward step procedures, they are somewhat unfounded and the significance of terms in the resulting model was therefore checked using a permutation test (Oksanen, 2008). The test adds terms to the model one by one in the order specified by the model formula, retaining previously added terms. Community (odonate

composition) data are permuted following the addition of each term, and the amount of inertia constrained by the model calculated. This is repeated a large number of times and the proportion of permuted constrained inertia values which are at least as large as observed constrained inertia represents the significance of that term.

Abundance estimates of odonate species at each site were $\log_{10}$ transformed before analysis. Variables measured in percentage cover were arcsine transformed. All explanatory variables were then standardised to zero mean and unit variance. All multivariate analyses were performed in R 2.4.0 (R Development Core Team, 2005) using the package Vegan 1.13-0 (Oksanen *et al.*, 2008).

3.2.3. *Ischnura pumilio* habitat associations

Generalized linear models (GLM) were used to assess which variables explained a significant proportion of variation in *I. pumilio* numbers. GLMs can be used to model data with non-normal distributions, and have been used in many studies of the effect of habitat variables on species distributions including damselflies (e.g. Rouquette & Thompson, 2005; Strange *et al.*, 2007) and other insects (e.g. Strauss & Biedermann, 2005). A Poisson error structure and logarithmic link function were used as they are most appropriate for modelling count data. The logarithmic link function ensures fitted values are positive and Poisson errors account for integer data with variance equal to the mean. However, as *I. pumilio* distribution was aggregated, there was additional variation in the response (overdispersion) which was accounted for by using quasi-Poisson errors. This applies a scale parameter to the model (Pearson's χ^2 / df), such that the variance increases with the mean (Crawley, 2007).

The maximal model containing all variables was simplified using a backwards selection procedure. The least significant variables based on *F* tests on deletion were removed until all variables were significant at $P \leq 0.05$ (Crawley, 2007). All non-normally distributed variables were subjected

to an appropriate transformation (see Table 3.2). Due to the large number of potential explanatory variables, model building was performed in several stages. Continuous variables were fitted first (along with their quadratic terms to test for non-linear effects), in sets which ensured the maximal model did not have more than n/3 parameters (Crawley, 2007). The minimum adequate model at the end of each stage was used as the base for the following stage of model building. Categorical variables were fitted in the later stages and when they remained in the minimum adequate model aggregated categories were also tested (Crawley, 2007). D^2 and adjusted D^2 (the equivalents to R^2 and adjusted R^2 in regression) were calculated using standard formulae (Guisan & Zimmermann, 2000).

Several variables were removed from the set of potential explanatory variables due to collinearity. This was determined by examining the covariance matrix of the continuous explanatory variables. Pairs of variables with correlation coefficient (r) values greater than the critical value of 0.463 ($df = 28$, $P < 0.01$) were deemed to be significantly correlated. A Bonferroni correction for multiple tests was not applied, as with 630 comparisons this would result in rejecting many correlated pairs. Variables removed from the analysis were geographical location (eastings and northings), altitude, percentage cover of water, conductivity, maximum height of vegetation and structural indices for zones 2, 5 and 10m. These were removed due to correlation with many variables, or in order to retain variables of greater interest. Despite removal of these variables, collinearity may still have had an effect on model selection as Graham (2003) states that r values as low as 0.28 may result in significant predictors being omitted from the final model. However, such extreme criteria would have prohibited model selection altogether. The criteria used were therefore chosen to allow production of a relatively robust model for comparison to those produced for other damselfly species, which used similar methods but did not consider collinearity (*Coenagrion mercuriale*, Rouquette & Thompson, 2005; *Ceriagrion tenellum*, Strange *et al.*, 2007).

In a separate GLM analysis, the effects of the various plant groups on *I. pumilio* abundance were considered. Before proceeding with this analysis, some groups were combined to remove collinearity (see Table 3.2). Plant cover data were arcsine transformed and fitted to a GLM model of *I. pumilio* abundance as above. The effect of other odonate species on the abundance of *I. pumilio* was considered in the same way by using $\log_{10}$ transformed counts of all other species as predictors.

3.2.4. Habitat use

The boundary between the areas adjacent to water and surrounding vegetation was distinct at Latchmoor, due to grazing and poaching by New Forest cattle and ponies which maintained waterside vegetation at a very low level (hereafter referred to as water area). Adjacent vegetation (hereafter referred to as hinterland – the area behind that lying along the shore of a river) was considerably taller comprising mostly bracken (*Pteridium aquilinum*) and shrubs (*Myrica gale* and *Ulex europaea*) with shorter tussocks of *Erica tetralix, Calluna vulgaris* and graminoids. The water-hinterland boundary was mapped in ArcView 9.2 using GPS records taken at approximate 10m intervals. In order to consider differing habitat use between sexes and the effect of weather, individual capture events were assigned to either the water or hinterland area. The proportion of individuals captured in the water area was then analysed using a GLM with quasi-binomial errors (due to overdispersion) with sex and weather as explanatory variables. Only the first mature capture of each individual was used. This avoided pseudoreplication arising from repeated observations of the same individual and additional error due to increased foraging behaviour in surrounding vegetation by immature individuals.

The minimum distance from water of each capture within this subset was also calculated using the multiple minimum distance tool extension (Chasan, 2005) for ArcGIS 9.2. The $\log_{10}$ transformed distances from water were used in an ANCOVA with the same explanatory variables. Time of day was included as a covariate including a quadratic term to test for any

effect of travelling from or returning to roosting sites at the beginning and end of the day. The effect of time was not considered in the previous analysis as the necessary sub-setting would have resulted in very sparse data.

Due to collinearity between weather measurements, a principal components analysis was performed on the 6 variables (maximum temperature, minimum temperature, rainfall, wind speed, cloud cover and solar radiation) obtained from local Met Office weather stations at Ibsley and Hurn (British national grid references SU162094 and SU117978 respectively; UK Meteorological Office, 2006). The first two principal components from this analysis accounted for 67% of the variance in weather conditions and formed the weather variables in the habitat use analyses (Graham, 2003; Knell, 2005). The first was strongly positively correlated with cloud cover and strongly negatively correlated with solar radiation and the second axis was strongly negatively correlated with maximum and minimum temperatures. Wind and rain were less correlated with the first two principal components, although both exhibited a positive relationship with PC1. Both principal components can therefore be thought of as representing good weather when negative and poor weather when positive.

3.3. Results

3.3.1. Site description

Ischnura pumilio was recorded at 17 of the 31 sites surveyed (Table 3.2). Many of those without sightings had changed significantly from the site description accompanying the past record, mostly becoming overgrown. NVC communities varied greatly, from aquatic communities (A24) to woodland and hinterland communities (W1, W4 and W25). Mires were the most common NVC type identified and accounted for 11 of the 17 sites with *I. pumilio,* mostly those in Devon and Hampshire. Of the four mire communities associated with *I. pumilio* presence, the most common was *Molinia caerulea-Potentilla erecta* mire (M25) followed by *Juncus effusus/acutiflorus-Galium palustre* rush-pasture (M23). Goodness of fit values were generally low (range 7 – 56 out of 100) and are all regarded as poor or very poor (Hill, 1996). This may be due to the sampling design inherently capturing a long moisture gradient and therefore different sub communities within sites. Most sites were shallow flushes or boggy areas with slow-flowing or still water, and a silt or silt and gravel substrate. *Juncus bulbosus* was present at every site where *I. pumilio* was recorded and *Juncus effusus* at all except one. The most commonly occurring plants at *I. pumilio* sites are listed in Table 3.4.

Table 3.4. Plant species occurring in 10 or more *I. pumilio* sites, number of occurrences in all 31 sites and in the 17 *I. pumilio* sites.

Species	All sites	*I. pumilio* sites
Juncus bulbosus	30	17
Juncus effusus	29	16
Juncus articulatus	22	14
Erica tetralix	21	13
Festuca rubra	23	13
Lotus pedunculatus	21	13
Ulex europaea	23	13
Calluna vulgaris	25	12
Potamogeton polygonifolius	18	12
Holcus lanatus	17	11
Potentilla erecta	17	11
Agrostis canina	14	10
Molinia caerulea	18	10
Viola sp.	15	10

3.3.2. Odonate habitat associations

In total, 27 variables were selected at least once during the first stage of analysis (Table 3.1). All of these variables could not be considered together in the second stage (due to insufficient df); therefore those selected three or more times in the 20 initial analyses were considered first. GPS northing values were removed from the model due to correlation with eastings, and when fitted separately eastings had a lower AIC value. Forwards and backwards selection of this model resulted in the same model specification. Each of the variables selected once or twice in the first stage were then added to the model one by one provided they did not cause overfitting with existing variables (indicated by VIFs > 10). Those resulting in a decrease in AIC were added to the model which was then subjected to a final stage of forwards and backwards selection. The best five models based on AIC are listed in Table 3.5.

Table 3.5. Model specification and AIC values for the best 5 CCA models of odonate species composition (log_{10} count data). Variable names are explained in Table 3.2.

Model	AIC
bankangle + conductivity + disturbance + easting + maxheight + MC + MM + muddepth + shade + spcount + TD + TFO + TG + waterdepth + z00 + z10 + z20	29.81
bankangle + conductivity + disturbance + easting + maxheight + MC + MM + shade + spcount + TD + TFE + TFO + TG + waterdepth + z00 + z10 + z20	30.1
bankangle + conductivity + disturbance + easting + maxheight + MC + MM + shade + spcount + TD+ TFO + TG + waterdepth + z00 + z10 + z20	30.56
bankangle + conductivity + disturbance + easting + maxheight + MC + MM + muddepth + shade + spcount + TD + TFE + TG + waterdepth + z00 + z10 + z20	32.25
bankangle + conductivity + disturbance + easting + maxheight + MC + MM + muddepth + shade + spcount + TD + TG + waterdepth + z00 + z10 + z20	32.39

In total, 81% of the variation in species composition was accounted for by the final model. All terms in the final model were analysed simultaneously using a permutation test. Due to sequential addition of terms, alternative sequences generated slightly different significance levels of some variables. However, most changed very little and the order of significance of variables was generally maintained. All terms in the best model based on AIC were

significant in at least one of the five sequential permutations tests conducted. Table 3.6 presents the results of the test performed with terms arbitrarily added in the order they appear in Table 3.2. Inevitably, several models will describe the data similarly well. However, the most significant terms in Table 3.6 were highly significant in all models and are likely to be the most important.

Table 3.6. Significance of predictors of odonate composition derived from a minimum adequate CCA model based on AIC. For each significant variable df, constrained inertia (χ^2) and the P-value derived from permutation tests (number of permutation = 9999) are shown (* P<0.05, ** P<0.01, *** P<0.001).

Variable	Df	χ^2	P
Easting	1	0.1101	***
Shade	1	0.1251	***
TD	1	0.0617	***
Disturbance	1	0.0382	**
Maxheight	1	0.0389	**
MM	1	0.0411	**
Structure zone 20	1	0.0442	**
Waterdepth	1	0.0394	**
Bankangle	1	0.0344	*
Conductivity	1	0.0298	*
MC	1	0.0341	*
Muddepth	1	0.0344	*
Spcount	1	0.0366	*
Structure zone 0	1	0.0346	*
TFO	1	0.0336	*
TG	1	0.0393	*
Structure zone 10	1	0.0275	.
Residual	12	0.1878	

The most important variables predicting odonate composition at a site were GPS location (east-west, although as GPS eastings and northings were highly correlated this may reflect latitude also), percentage cover of shade and the percentage cover of terrestrial dwarf shrubs. Also highly significant were level of disturbance, maximum height of vegetation, percentage cover of *Sphagnum* moss species, the level of structural complexity at 20m from water and water depth. Figure 3.2 shows an ordination diagram of the final model.

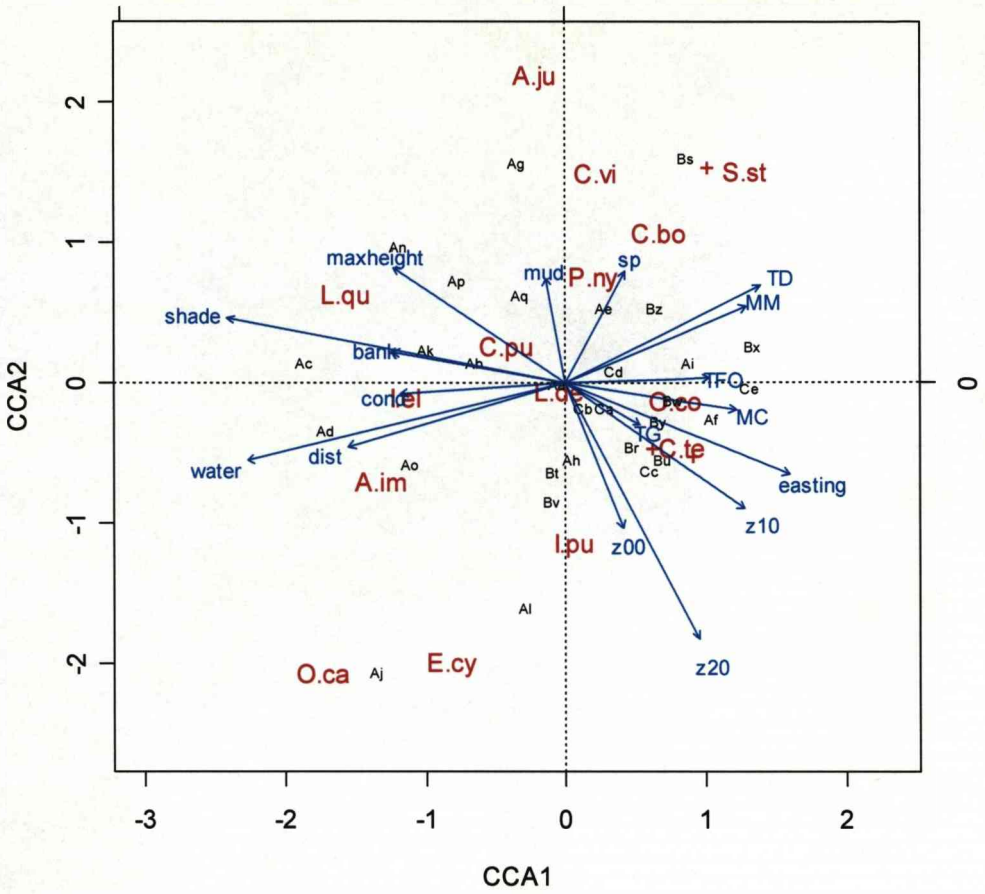


Figure 3.2. Ordination diagram of the final CCA model of odonate community composition. Species codes in red are explained in Table 3.3 and site codes in black in Table 3.1. Some variable names have been abbreviated to reduce overlap of labels, reduced names are explained in Table 3.2. Where species codes overlap, the less abundant species is represented by "+". The "+" adjacent to S.st represents S.da and those adjacent to C.te represent L.sp and C.me. Arrows represent the direction of the gradient of significant predictors. Sites and species are scaled symmetrically by the square root of eigenvalues (Oksanen *et al.*, 2008).

In an ordination plot, the sites which have the most similar species composition are plotted closest together. Similarly, species which frequently co-occur appear close together, and the species common within a particular site are plotted close to that site. The arrows represent the direction of increase in measured environmental variables. Sites with deep water and species preferring deep water will therefore appear at the side of the plot to which the water depth arrow (labelled "water" in Figure 3.2) points. The difference in arrow lengths is the result of a multidimensional solution being plotted in two dimensions and is not indicative of the strength of correlation (Oksanen, 2008). This plot shows only the community structure related to the environmental variables in the final model. However, the similarity of

the relative positioning of sites and species to those in Fig. 3.1 suggests that omitting community structure not related to measured environmental variables was justified.

Orthetrum cancellatum, *E. cyathigerum* and to a lesser degree *Anax imperator* formed a group to the left of the plot, all of which are associated with large water bodies and can tolerate brackish, eutrophic or mineral rich water (Smallshire & Swash, 2004). The arrows show that these species were associated with deeper water and higher levels of disturbance, which were common features of the clay mining sites surveyed. *Aeshna juncea*, *Calopteryx virgo, Sympetrum striolatum, S. danae* and *Cordulegaster boltonii* formed another group, all preferring acid water (Smallshire & Swash, 2004). Although pH was not included in the analysis, this was supported by the association with *Sphagnum* moss species which are a common feature of acid bogs and mires. Along with *Pyrrhosoma nymphula*, which has broader habitat requirements, this group was associated with greater odonate species richness. *Libellula quadrimaculata* also prefers acidic habitat and appears somewhat associated with the previous group, but was not observed outside Cornwall and is therefore plotted at a lower easting value. In the centre of the plot are the species with broad habitat requirements but preferring smaller sites with standing water such as *Libellula depressa, Coenagrion puella* and to a lesser degree *Ischnura elegans* which is less common in acidic waters and is plotted further from those species preferring acid conditions.

Lestes sponsa, *Ceriagrion tenellum* and *Coenagrion mercuriale* were very closely associated, and *O. coerulescens* was plotted in the same area. These species are all associated with heathland sites characterised by bog mosses (as indicated by the *Sphagnum* arrow labelled MM) except *C. mercuriale* which is more specific in its requirements preferring base rich, open, slow flowing waters (Smallshire & Swash, 2004). *Ischnura pumilio* was most associated with this group, also preferring small, open and shallow water bodies, but tolerating a wide variety of water quality conditions. However, *I. pumilio* was plotted relatively far from any other species, suggesting that it has different habitat requirements to all other species considered.

3.3.3. *Ischnura pumilio* habitat associations

Ischnura pumilio was plotted centrally on the first CCA axis which represents the major environmental gradient influencing species composition. This axis was correlated with eastings, so that sites in Cornwall were mainly plotted on the left and those in Devon and Cornwall on the right. Also correlated with this axis were shade, water depth, disturbance, maximum vegetation height, bank angle, conductivity and the percentage cover of several plant groups. *Ischnura pumilio*'s central placing indicates that these factors do not strongly influence its distribution. The positioning of *I. pumilio* at a low value along the second CCA axis suggests the species prefers shallow mud, a low species count and increased vegetation structural diversity.

Table 3.7. Significant predictors of numbers of *I. pumilio* at a site derived from a generalized linear model with quasi-Poisson errors. The *F*-value and associated P-value, df, D^2 and adjusted D^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates on the logarithmic scale and standard errors (SE) are shown (* P<0.05, ** P<0.01, *** P<0.001).

Model summary	Variable	Parameter Estimates	SE	*t*	P
Deviance = 63.903	Mud cover	4881.00	1150.00	4.248	***
df = 19	Mud cover^2	-1.22 x10^6	2.79x10^5	-4.361	***
Dev/df = 3.36	Shade	27710.00	5510.00	5.026	***
D^2 = 0.901	Shade^2	-4.04 x10^7	8.42x10^6	-4.801	***
Adj. D^2 = 0.850	Substrate - silt	3.623	0.91	3.971	***
	Type – flush	-2.223	0.51	-4.365	***
	Structure z20	0.9387	0.31	3.046	**
	Turbidity	21.04	8.46	2.488	*
	Turbidity^2	-8.369	3.25	-2.572	*
	Area	0.5685	0.32	1.777	.
	Intercept	-1.87E+01	5.39	-3.473	**

GLM analysis supported the results of the CCA analysis in terms of *I. pumilio* habitat preferences. Sites with low levels of shade and mud coverage were favoured by *I. pumilio*, although absence or very low levels of shade or mud were associated with a decrease in numbers as indicated by the quadratic term (Table 3.7). Substrates consisting mainly of silt were preferred over gravel, and bog habitats were preferred over flushes. Increased structural complexity at 20m from water was associated with an increase in numbers. However, vegetation structure at the water did not

feature in the final model, despite being closely associated with *I. pumilio* in the CCA plot. Numbers were greater in water with moderate to high turbidity ratings which may be linked to finer substrates being suspended in water, but at the highest levels *I. pumilio* abundance decreased. A positive association with area was marginally significant and an *F* test on deletion indicated a significant decrease in explained variation, therefore the term was retained.

When odonate species abundances (log_{10} transformed) were used as predictors in a separate GLM analysis of *I. pumilio* abundance, three species were found to be significant predictors (Table 3.8). *Orthetrum coerulescens* and *I. elegans* were positively associated with *I. pumilio*, and *Cordulegaster boltonii* negatively associated. A negative association with *Libellula quadrimaculata* was marginally significant and an *F* test on deletion indicated a significant decrease in explained variation, therefore the term was retained. However, when these predictors were added to the overall model they were non-significant and were removed by stepwise deletion. Habitat features were therefore better predictors of *I. pumilio* abundance than the estimated abundance of any of the other recorded odonates.

Table 3.8. Significant predictors of numbers of *I. pumilio* at a site derived from a generalized linear model with quasi-Poisson errors. The *F*-value and associated P-value, df, D^2 and adjusted D^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates on the logarithmic scale and standard errors (SE) are shown (* P<0.05, ** P<0.01, *** P<0.001).

Model summary	Variable	Parameter Estimates	SE	*t*	P
Deviance = 321.02	*C. boltonii*	-5.219	2.036	-2.563	*
df = 25	*O. coerulescens*	1.335	0.490	2.728	*
Dev/df = 12.84	*I. elegans*	1.177	0.512	2.298	*
D^2 = 0.505	*L. quadrimaculata*	-2.531	1.271	-1.990	.
Adj. D^2 = 0.425	Intercept	0.513	0.864	0.594	

Due to severe multicollinearity both between plant groups and with other habitat variables, a separate GLM analysis of the effect of plant groups was conducted in order to attempt to identify the important groups for consideration in the general model. However, no significant effects were found. There may be several reasons for this, including collinearity causing

omission of significant variables (Graham, 2003). However, it may be expected that *I. pumilio* abundance will not depend on the cover of broadly defined plant groups. Within these groups plants will have different habitat requirements, and if an association with a species were based on common requirements these would remain undetected due to the groupings. Furthermore, *I. pumilio* is very broad in the range of habitats it can occupy, particularly with respect to chemical factors which are likely to influence plant community.

It was expected that associations with plant groups may be based more on structural features than common habitat requirements. However, many different plants can provide the same structural characteristics and therefore species or even groups may not be important. *I. pumilio* seems more dependent on features of the site and substrate than specific vegetation and other odonates. Observations during field work suggested that important vegetation features were emergent vegetation for oviposition and male perching; and tussocks of graminoids and heathers for shelter during windy conditions and presumably at night also. This is supported by the proportion of species listed in Table 3.4 as frequently co-occurring with *I. pumilio* which are graminoids or heathers (62%).

3.3.4. Habitat use

There was an overall increase in distance from water in poor weather (Table 3.9). Distance of captures from water increased with increasing cloud cover and decreasing solar radiation (PC1). Lower maximum and minimum temperatures were associated with greater distances from water in female *I. pumilio*, but had virtually no effect on the relative location of males (PC2). Time of day had no direct effect on distance from water, but later captures exhibited a stronger positive correlation between PC2 and distance. There was a significant increase in the proportion of recorded females to males with time of day ($P < 0.0001$, $D^2 = 0.625$; Fig. 3.3) which may have caused the increased correlation between PC2 and distance later in the day as females exhibited this trend more than males. Overall the back transformed mean distance of males from water predicted by the model (5.95m) was less

than that of females (6.53m) although this is likely to be an underestimate of the difference, as a large number of females inevitably went undetected away from water due to their greater crypsis.

Table 3.9 Significant predictors of distance from water (log_{10}) derived by multiple regression. The *F*-value and associated P-value, df, R^2 and adjusted R^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates and standard errors are shown (* P<0.05, ** P<0.01, *** P<0.001).

Model summary	**Variable**	**Parameter Estimates**	**SE**	***t***	**P**
$F = 11.57$	PC1	0.050	0.008	6.414	***
P = ***	PC2	-0.187	0.073	-2.558	*
df = 1117	Sex	0.054	0.024	2.233	*
$R^2 = 0.059$	Time	0.143	0.124	1.153	
Adjusted $R^2 = 0.053$	PC2:Sex	0.063	0.025	2.544	*
	PC2:Time	0.305	0.121	2.517	*
	Intercept	0.730	0.077	9.485	***

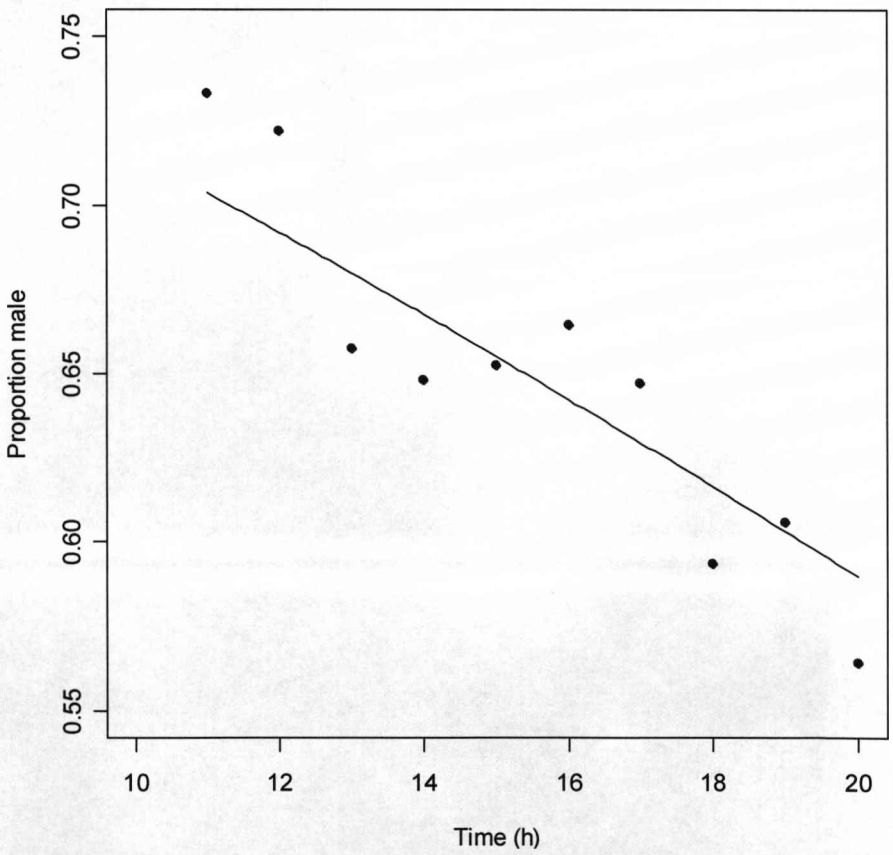


Figure 3.3. Relationship between the proportion of captures which were males and time of day. Time values are measured on the 24 hour clock and points include all captures made in the hour preceding the plotted time ($D^2 = 0.625$, adjusted $D^2 = 0.578$).

The proportion of males captured in the area adjacent to water compared to hinterland (0.575) was significantly greater than that of females (0.481). However, this relationship varied significantly with temperature (Fig. 3.4); the proportion of females captured at water decreased markedly at lower temperatures (PC2), whereas that of males increased slightly. The relationship was very similar to that between distance from water and temperature (PC2) in males and females, and for this reason only one figure is included. There was no significant effect of cloud cover and solar radiation (PC1) in this analysis, suggesting that temperature was the most important weather element influencing the location of *I. pumilio*. The results of this analysis are summarised in Table 3.10.

Table 3.10 Significant predictors of the proportion of individuals captured in the area adjacent to water derived from a generalized linear model with quasi-binomial errors. The *F*-value and associated P-value, df, D^2 and adjusted D^2 are shown. For each significant variable the P-value derived from *t*-tests, parameter estimates on the logit scale and standard errors (SE) are shown (* P<0.05, ** P<0.01, *** P<0.001).

Model summary	Variable	Parameter Estimates	SE	*t*	P
Deviance = 138.86	PC2	0.07227	0.0779	0.928	
df = 76	Sex	-0.38467	0.17212	-2.235	*
Dev/df = 1.83	PC2:Sex	-0.52105	0.18215	-2.861	**
D^2 = 0.109	Intercept	0.30125	0.09192	3.277	**
Adj. D^2 = 0.056					

The proportion of variance in the response variables explained by these models is small (R^2 = 0.059 and D^2 = 0.109 respectively). Evidently there were significant factors influencing an individual's location in relation to water which were not measured. The need to forage is likely to cause individuals to venture further from water and as examining gut contents was not possible this could not be accounted for. Also, females only visit water when ready to mate (Banks & Thompson, 1987; Anholt, 1992; Stoks, 2001a, b) and similarly this could not be accounted for by examining ovaries for mature eggs.

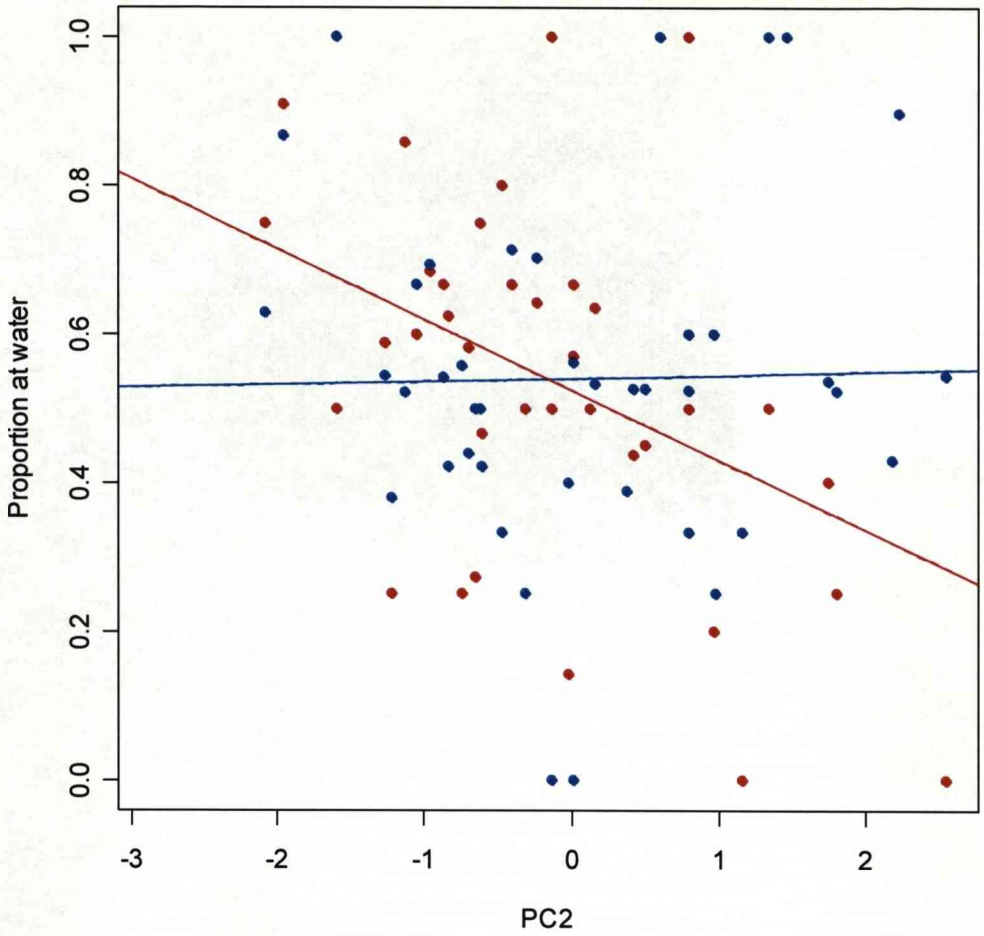


Figure 3.4. Relationship between the proportion of individuals captured in the area adjacent to water and the second principal component of weather variables representing lower temperatures at higher values ($D^2 = 0.109$, adjusted $D^2 = 0.056$).

3.5 Discussion

3.5.1. Odonate habitat associations

Shade was highly important in predicting odonate composition in this study. This was also reported in a previous study, which found most anisopterans were restricted to sunnier biotopes (Samways & Steytler, 1996). Remsburg *et al.* (2008) also found strong evidence for shade avoidance in anisopterans at 75% and 30% shade cover. Anisopterans were found at all levels of shade within the sites in this study, however, as all of these sites were relatively open, the total shade range surveyed is unlikely to include sufficiently high levels to exclude these species. Vegetation height and structure was also highly influential on odonate species composition. Structure of vegetation was concluded to be more important than species in predicting odonate composition (Samways & Steytler, 1996) and composition of other insect taxa (e.g. aphids; Strauss & Biedermann, 2005), where it was suggested to be an indirect measure of a site's microclimate. This may also be true in this study, as sparse vegetation causes more extreme temperature conditions and provides less shelter from wind, rain and predators. However, Schaffers *et al.* (2008) found plant species composition to be a better predictor of arthropod species composition for several groups, although many were specialised herbivores which may be expected to rely heavily on particular plant species. As adult odonates are generalist predators of insects (Corbet, 1999) and utilise plants solely for perching and roosting, structural features are likely to be of greater importance.

Within the range of habitats surveyed, there was a negative relationship between structural diversity of vegetation (measured up to 1.5m) and the maximum height of vegetation and corresponding shade cover (Fig. 3.2). This may be interpreted as a distinction between early-successional sites which have abundant, tussocky low-level vegetation, but little to no taller vegetation; and more mature sites which have more shrubs and trees that overshade low-level vegetation, changing its composition and decreasing density. Damselflies were mostly associated with the denser, low-level

vegetation, although *P. nymphula, Coenagrion puella* and *Calopteryx virgo* were intermediate. The larger dragonflies were mostly associated with less structurally diverse vegetation with a greater maximum height. Damselflies are generally restricted to one site, where significant over-shading will reduce their ability to warm up and thus negatively affect foraging and mating behaviour (Remsburg *et al.*, 2008). As dragonflies are more mobile than damselflies, they may visit several sites in a day and therefore be less affected by increased shade at certain sites, visiting them only briefly for foraging or mate searching.

Other than shade and percentage cover of exposed macrophytes, the important predictors of odonate composition in this study were different to those found by Samways & Steytler (1996). Water temperature was important in their study, but due to missing data could not be included in this analysis. Flow rate featured in their analysis both directly and indirectly through amount of exposed rock, but was not significant in this study. Again, this may be due to the small range of flow rates observed as a result of concentrating on sites previously inhabited by *I. pumilio*, which is known to prefer slow-flowing water (Cham, 1991; Smallshire & Swash, 2004).

Cornish habitat types were more varied than those in Devon and Hampshire, and as a result had more diverse species compositions. This is demonstrated by the spread of Cornish sites and associated species around the periphery of the ordination plot, and by the dominance of one half by only Cornish sites. Sites in Devon and Hampshire were plotted more closely and associated with some of the most common species in the study *I. pumilio* and *O. coerulescens*. Also associated with these sites and species were the less commonly observed species *Ceriagrion tenellum*, *Lestes sponsa* and the nationally rare *Coenagrion mercuriale*. Increased marginal *Carex* and *Sphagnum* species and terrestrial grasses, flowering plants and dwarf shrubs characterised these sites; along with low levels of shade, shallow bank angles, shallow water, low maximum vegetation height and increased vegetation structural diversity. Rouquette & Thompson (2005) found that *C. mercuriale* was associated with wide underwater berms (resulting in shallow

underwater ledges), bankside monocots and the absence of trees (attributed to shade avoidance) which is in accordance with the findings of the CCA.

Coenagrion mercuriale was not recorded at all in Cornwall and along with *Ceriagrion tenellum* and *L. sponsa* (recorded twice and once in Cornwall respectively), clearly prefers the more homogenous habitats found further east. The ordination plot indicates a few Cornish sites which are similar to those inhabited by this rare species, and which may be considered if a *Coenagrion mercuriale* reintroduction program were planned. Those sites are Chapel Porth 2, Peter's Point and Wimal Ford. However, of these sites, only Wimal Ford is large enough to support a reasonable breeding population and given the relative rarity of suitable habitat to disperse to in Cornwall, such a program may not be successful.

The most commonly observed damselfly species were the nationally common and eurytopic *P. nymphula, Coenagrion puella* and *I. elegans*. However, the most commonly observed dragonfly was *O. coerulescens,* which is nationally much less common and has more specific habitat requirements. The species has a similar south-west distribution in the UK to *I. pumilio* (Smallshire & Swash, 2004) and is frequently observed alongside it in Cornwall (S. Jones, pers. com.). The results of the GLM indicate that it is associated with *I. pumilio,* and its greater frequency of occurrence suggests that *O. coerulescens* can persist at a site after succession has excluded *I. pumilio*.

3.5.2. *Ischnura pumilio* habitat associations

In this study, *I. pumilio* preferred sites which were somewhat muddy with silt rather than gravel substrates. This is characteristic of slow flowing or standing water where silt can accumulate without being carried away by the current. A preference for some turbidity is also linked to these habitat features as small particles become suspended in water, particularly where poaching by livestock or other disturbance takes place. Grazing and disturbance did not feature in this model although they are often said to be

important factors (Fox, 1987; 1989; Cham, 1991). However, the openness of some *I. pumilio* sites was maintained in other ways. High soil mineral levels from previous tin mining resulted in sparse and unusual vegetation communities at sites such as Great Wheal Seton. A degree of bare ground is known to be favoured by the species (Fox *et al.*, 1992) and it is openness, rather than the method of maintaining it, which is likely to be important to *I. pumilio,* as indicated by a decrease in numbers where there was no bare ground.

The CCA analysis (Fig. 3.2) showed that *I. pumilio* was associated with increased structural diversity but low maximum vegetation height and also low odonate species counts. This is characteristic of the early-successional sites known to be preferred by the species (Fox, 1989; Daguet, 2005), where low-level vegetation may be dense before larger, over-shading plants have colonised. This was supported by the GLM results, which indicated a preference for low levels of shade which may be associated with low vegetation and an absence of trees. Remsburg *et al.* (2008) found that shade alone reduced dragonfly numbers in *Trithemis* species, and it was negatively associated with abundance of those species close to *I. pumilio* in Fig. 3.2. Increased structural diversity of vegetation provides greater shelter from wind, rain and predators, and *I. pumilio* individuals were regularly observed dropping down into tussocks of vegetation when approached on cooler, windier days. This was an effective defence when conditions were perhaps too cool to fly away, as these individuals were subsequently very difficult to find. The base of such tussocks is likely to represent a sheltered microclimate, with a low risk of being damaged by wind in the dense, soft grass stems. Tenerals and adult females have been said to take shelter in thick scrub formed by bog myrtle (Fraser, 1941), however sheltering individuals were never observed on shrubs. Rouquette & Thompson (2007b) reported that the majority of *C. mercuriale* roosted on tussock forming *Juncus inflexus* and *Deschampsia cespitosa,* preferring grassy areas to forest edges or other linear features. Askew (1982) suggested that coenagrionid damselflies select roosting perches which are very similar in width to the distance between the individuals eyes. This was said to allow surveillance

whilst maintaining camouflage. Observations during this study support this hypothesis, as *I. pumilio* individuals were observed rotating around a perch in order to position it between them and the observer; a behaviour which has also been observed in larval stages (Fraser, 1949).

The plant species most frequently co-occurring with *I. pumilio* (Table 3.4) were somewhat different to those listed by Fox (1987, 1989) and Cham (1990), featuring only one common species: *Juncus effusus*. However, *Ranunculus flammula* listed by Fox (1987) was recorded at 8 of the 17 *I. pumilio* sites and a female was observed ovipositing into this species. It is unlikely that particular plant species are preferred by damselfly species, but they may either indicate favourable habitat or have soft stems suitable for oviposition (Rouquette & Thompson, 2005 and refs therein). Associations with plant species could not be more quantitative than this due to the large number of plant species identified and the sparse distribution of many, which could not have been normalised before analysis. Plants were therefore combined into functional groups which reduced the problem of collinearity common with many predictor variables. However, this resulted in no significant effects which may be due to loss of detail by grouping.

Ischnura pumilio was not absent from sites where *Sphagnum* moss was recorded but, as suggested by Fox (1987), was absent from those sites dominated by this species. *Sphagnum* species commonly dominate acid bogs and wet heaths and indicate a low pH. *Ischnura pumilio* was previously thought to prefer base-rich water, and dislike acidic sites (Fox, 1987). Due to missing data values pH could not be included in the analyses but *I. pumilio* was recorded at sites with pH values ranging from 4.4 to 7.2. The lowest pH recorded was within the range characterising acid heathland (3.4 – 6.5; Price, 2003), suggesting that *I. pumilio* can occur in a wider range of habitat types than previously thought.

The variety of NVC types inhabited by *I. pumilio* is surprising (Table 3.1). *Coenagrion mercuriale* has a similar distribution in the UK, although less common, and is restricted to only a few NVC types. Purse & Thompson (2009) found that the habitat of *C. mercuriale* at Upper Crockford, New

Forest represented five NVC communities (S19, *Eleocharis palustris* swamp; M21, *Narthecium ossifragum-Sphagnum papillosum* valley mire; M15; M25 and M29 definitions provided in Table 3.1). Furthermore, the species is known to be restricted to two specific habitat types throughout the UK; small, base-rich, lowland, heathland streams and calcareous streams and fens. The nine NVC communities with *I. pumilio* presence in this study fall into six major NVC vegetation types, compared to only two for *C. mercuriale*. This supports the findings of the analyses above that vegetation type and the underlying factors controlling plant communities such as pH and conductivity do not significantly affect the suitability of a site for *I. pumilio*. It is interesting to note that although *I. pumilio* and *C. mercuriale* are often associated with heathland (Smallshire & Swash, 2004; Rouquette & Thompson, 2005), neither this study nor Purse & Thompson (2009) classified any vegetation type as heath as defined by NVC, both species appearing to prefer mire communities.

Water depth could not be included in the analysis due to collinearity and as measurement of depth at the centre of the water was not possible at some sites. However, as all but two sites were previously inhabited by *I. pumilio*, it is unlikely that the range of depths was great enough to detect an effect, as bank angle and depth (irrespective of the water table) are unlikely to change. *Ischnura pumilio* is also known to be able to tolerate fluctuating water levels to the point of complete drying out for short periods (Cham, 1991; Fox *et al.*, 1992; Cham, 1992a); suggesting that within the range of shallow waters surveyed, depth is unlikely to be important. This is supported by the fact that *I. pumilio* was recorded at the deepest water bodies in this analysis, albeit in small numbers, some of which were large pools that may have been several metres deep at the centre. This contradicts previous notions that *I. pumilio* is restricted to shallow, spring-fed water courses due to thermal requirements (Cham, 1991; Fox & Cham, 1994; Strange *et al.*, 2007).

As almost all of the sites surveyed had previously supported *I. pumilio* populations, those factors selected as predicting greater abundance may be considered those which are required for its persistence at a site. Absence of

I. pumilio from sites with past records is likely to be due mostly to those sites becoming overgrown and over-shaded. This was evidenced by a preference for some bare ground near water (mud cover), also observed previously (Cham, 1991; Fox & Cham, 1994), and a low level of shade. Increased odonate species count was associated with the absence of *I. pumilio* in the CCA analysis, which may be coincidental due to changing habitat conditions, or may be due to competition for resources, interspecific aggression or predation of *I. pumilio* by larger species. Similarly, the negative association with *Cordulegaster boltonii* and *Libellula quadrimaculata* may be due to predation by these species or to their preference for acidic conditions and in the case of *C. boltonii,* fast flowing water. A positive association with *C. boltonii* has been previously suggested (Fox, 1987) but seems unlikely given their different habitat preferences. However, large dragonflies such as *C. boltonii* may cover large areas every day, and in so doing may visit areas preferred by *I. pumilio,* particularly where they are close to faster running water such as at Latchmoor. *Libellula depressa* was not associated with *I. pumilio* as might be expected given its tendency to colonise newly formed ponds and mineral workings, in fact the species was more common where *I. pumilio* was absent than where it was present (5 and 4 observations respectively).

The relative closeness of *Coenagrion mercuriale* and *Ceriagrion tenellum* to *I. pumilio* on the ordination plots, suggesting frequent co-occurrence, is in accordance with the observations of Fraser (1941). The plots also suggest that *I. pumilio* is equally associated with *Lestes sponsa*, at least in terms of its preference for the measured environmental variables. Despite the associations discussed here, the GLM revealed that the significant environmental variables identified are better predictors of *I. pumilio* abundance than other odonate species.

Although no direct measurement of pollution or water quality was made in this study, it was apparent from the variety of conditions supporting *I. pumilio* populations that water quality was not of major importance. Figure 3.5 illustrates the range of conditions in which *I. pumilio* was recorded. An

element of pollution at a site may not exclude *I. pumilio* as previously suggested (Fox & Cham, 1994; Daguet, 2005) but may in fact favour the species by exclusion of other odonates, or suppression of vegetation maintaining an early-successional habitat type such as that at Great Wheal Seton and other Cornish ex-industrial sites (Jones, 1985). The records of *I. pumilio* from motorway storm-water retention ponds, with high concentrations of copper and zinc in the top sediment layer and herbicides in the water column (Scher & Thièry, 2005), also support the idea that the species can tolerate considerable levels of pollution.

Figure 3.5. From top left to bottom right: Great Wheal Seton, Cornwall, the most visibly polluted I. pumilio site; close up of water at Great Wheal Seton; Stepper Point, Cornwall, a coastal site with the highest conductivity of those with I. pumilio records in this study; Latchmoor, New Forest, a relatively undisturbed, unpolluted stronghold site for I. pumilio.

The two sites considered ideal *I. pumilio* habitat which had no past records were Wimal Ford and Newlyn East Downs (S. Jones, pers. com.). These sites had values mostly within the ideal range for any of the significant predictors of *I. pumilio* habitat but colonisation by the species was not recorded. Newlyn East Downs had deeper mud than most *I. pumilio* sites, which could not be included in the GLM due to collinearity. However, it seems unlikely that mud depth would be highly influential as neither larvae

nor adults utilise mud *per se*. Neither site is particularly isolated relative to other *I. pumilio* sites and both appear suitable.

Development of effective conservation strategy requires detailed knowledge of the habitat requirements of all life stages (Thompson *et al.*, 2003). Therefore, the findings of this study cannot represent the full range of habitat requirements of *I. pumilio* or the other species considered. Larval habitat is potentially more important than adult habitat as larvae are confined to the water body (Thomas, 1994; Hardersen, 2008). Vegetation preferences may be stronger as plants provide shelter from predators (Thompson, 1987; Elkin & Baker, 2000) and are also important for foraging (Convey, 1988). Water temperature is also likely to be important for larvae (Hickling *et al.*, 2005) along with the physical conditions influencing it such as depth of water and over-shading. No study of larval habitat associations in *I. pumilio* has been undertaken to date, possibly due to the difficulty of distinguishing larvae and exuviae from those of the co-occurring *I. elegans,* although reliable methods are available (S. Jones, pers. com.). Additionally, Fraser (1949) noted that *I. pumilio* nymphs were difficult to dredge due their concealment in vegetation.

3.5.3. *Ischnura pumilio* habitat use

The importance of habitat away from water was emphasised by the findings of this study. Fifty-two percent of females were captured in vegetation away from the immediate water area on average and this increased during poor weather. Similarly the distance of females from water increased during poor weather whereas male distance changed very little. This indicates that females spend more of their time foraging and sheltering in vegetation and move to water to mate only during favourable weather (Banks & Thompson, 1987; Anholt, 1992; Stoks, 2001a, b). In a study of *Somatochlora hineana*, Foster & Soluk (2006) found females spent more time away from breeding habitat and attributed this to avoidance of harassment by males. Harassment is costly to females in terms of energy expenditure, foraging time lost and

potential damage during forced mating attempts (Corbet, 1999; Foster & Soluk, 2006).

Males on the other hand were similarly likely to be captured at water regardless of weather. This may be expected as males should benefit from increased time at the water, by maximising the number of matings they are able to obtain. However, tandem and copulating pairs of *I. pumilio* were frequently observed in surrounding vegetation during good weather. The maximum recorded distance of a mating from water was 55m (maximum for any capture 101m) and 30% of matings were recorded at more than 10m from water. Whether mating pairs moved away from water once in copula or mating commenced there, this highlights the importance of the surrounding area for providing protection from displacement behaviour from other males during copulation.

Foster & Soluk (2006) observed that males may optimise their mating rate by seeking out females where they are most abundant. They attributed the absence of this behaviour in *S. hineana* to reduced reproductive success in males mating away from water, due to females re-mating when returning to water once fully receptive. Both dragonflies and damselflies have mechanisms which ensure the last male to mate with a female has a high proportion of the fertilisation precedence when oviposition takes place immediately after mating (Corbet, 1999). A female leaving the hinterland to mate at water is likely to be receptive and ready to oviposit immediately, reducing the sperm precedence of males from previous matings in the hinterland. However, *I. pumilio* was frequently observed mating away from water, which may result in reduced reproductive success. One explanation for this is that pairs move away from water after commencing copulation in order to avoid displacement behaviour by other males. Alternatively, some males may remain in the hinterland or visit it periodically, mating with any females they encounter and obtaining high fertilisation precedence in some of those matings by chance when the female is ready to oviposit.

It is common for MRR studies to capture more individuals at high temperatures, low wind speeds and low levels of cloud cover (e.g. Ward &

Mill, 2006) and as such most studies are conducted only during these conditions. All odonates require solar energy to reach a sufficient body temperature for flight. When this temperature is not reached, the animal will be unable to move away from its roosting location, resulting in the increased number observed in the hinterland during poor weather in this study. The effects of wind speed, cloud cover and other weather variables on odonate behaviour are all likely to be ultimately related to their effect on ambient temperature, with the additional risk of damage to individuals flying during strong winds (Ward & Mill, 2006). The protection from wind and low temperatures offered by the tussocky vegetation used for roosting was discussed in the previous section.

Other studies of odonates have found the area surrounding water to be important also. A study of *Coenagrion puella* and *I. elegans* at a group of ponds made 75% of all sightings more than 45m from water (Conrad *et al.*, 2002). In a survey of anisopteran species, Bried & Ervin (2006) found that mean abundance did not change with distance from water up to 160m. Furthermore, males were more frequent within 40m of water and females more so between 130 and 160m. Samways & Steytler (1996) recommended that a 20m riparian strip of indigenous shrubs between river and trees should be maintained to attract odonates such as *Chorolestes tessallatus* and *Cordulegaster boltonii* in South Africa. *Coenagrion mercuriale* chose roosting locations based on suitable vegetation rather than minimal distance from water, and were evenly distributed throughout 50×50m plots (Rouquette & Thompson, 2007b). The study also determined that diurnal perching vegetation was different to nocturnal roosting vegetation, suggesting that daytime surveys do not capture the entire range of habitat utilised by a species, and emphasised the importance of roosting vegetation. The width of an effective buffer will depend on the species or species assemblage in question. At Latchmoor only 1.1% of captures were over 50m from water, and only 5.8% over 25m from water. However, at sites with lower habitat quality, individuals may have to fly further to roost and it must be ensured that areas for roosting are maintained within a reasonable distance given the species movement capacity.

The increased proportion of captures which were female as the day progressed indicates that females become more active later in the day. In contrast, Ward & Mill (2006) found that activity of both male and female *Calopteryx splendens* exhibited two peaks at approximately 11am and 2pm. However, *I. pumilio* does not become active until late morning and remains active until mid evening (pers obs; Verdonk, 1979) unlike many other damselflies which commence and cease activity earlier in the day (Banks & Thompson, 1985b; Corbet, 1999; Ward & Mill, 2006). *Ischnura elegans* females are known to oviposit during the last few hours before sunset (Miller, 1987) and *I. pumilio* may behave similarly. Unfortunately, oviposition was witnessed very few times during both field seasons, which lends support to this hypothesis, as fieldwork ceased several hours before sunset. However, females are perhaps at their most cryptic during oviposition, as they were extremely hard to follow once identified ovipositing, due to their green colouration likening them to small emergent leaves and stems.

3.5.4. Summary

Threats to *I. pumilio's* persistence have been identified as continued mineral extraction at quarry sites; lack of habitat management; seral succession and scrub encroachment; water pollution; and disturbance of aquifer fed spring lines leading to loss of habitat (Daguet, 2005). However, it seems that *I. pumilio* can inhabit a broad range of habitat types. Jones (1985) noted that the species is able to survive in the potentially toxic waters of previous tin mining sites and the species persisted at one such site at least in Cornwall in 2006 (Great Wheal Seton). Continued mineral extraction may not present a problem provided new habitat is created by the process for colonisation. However, succession, scrub encroachment and lack of management allowing these processes to occur are all likely to be detrimental.

This study identifies habitat features which would assist the development of a conservation management plan for *I. pumilio*. Although neither grazing nor human disturbance were selected in the final model, they seem the ideal

method for maintaining the degree of openness required by *I. pumilio* (Fox & Cham, 1994). Grazing and poaching by New Forest cattle and ponies create the open, muddy areas characteristic of the best of the *I. pumilio* sites considered here, Latchmoor. Grazing also maintains the early-successional status of a site, and suppresses the growth of over-shading larger vegetation.

Ischnura pumilio was previously thought to have specific habitat requirements, based mostly on anecdotal evidence (Fox, 1987; 1989; Cham, 1990; 1991; 1992a; Fox & Cham, 1994). However, some of the features thought to be important did not feature in this study. The species was found at a range of water depths, management regimes and levels of pH, pollution, grazing and disturbance levels. However, as previously suggested the sites surveyed were generally slow-flowing, with some bare ground in and around the water's edge and relatively open in terms of shade from tall vegetation.

Before application of these findings to conservation projects, particularly at lower latitudes, it would be desirable to validate the models by applying them to data sets from other regions (Guisan *et al.*, 2002). As *I. pumilio* reaches the northern limit of its range in the UK, the niches it is able to occupy may become rapidly narrower towards its range margin, as conditions become increasingly prohibitive. The pronounced ecological flexibility of *I. pumilio* has been attributed to its high chromosome number compared to the Coenagrionidae as a family, which increases the recombination index allowing an increased capability for ecological adaptation (Kiauta, 1979). Genetic analysis of the UK *I. pumilio* population will reveal the level of genetic distinction between regions, but genetically distinct populations may also have evolved different habitat selection (Whittingham *et al.*, 2007). Therefore these results may not be useful in other geographical areas. Transferability of habitat models has been demonstrated in other insects (e.g. Bonn & Schröder, 2001; Binzenhöfer *et al.*, 2005) but has not been attempted with odonate species. Unfortunately, the data set analysed here was too small to split for model building and validation.

Management of a habitat will depend on whether the priority is conservation of maximum number of species or a single species such as *I. pumilio*. Maintenance of vegetation which is low level but structurally diverse at sites with shallow water will favour less common species such as *I. pumilio*, *O. coerulescens* and the nationally rare *Coenagrion mercuriale*. *Ischnura pumilio* requires a low but present level of bare ground (Cham, 1991; Fox *et al.*, 1992), specifically shallow mud and is associated with low overall odonate species count. Management for other species or assemblages of species would require different criteria. It is clear that any designated conservation area should include the adjacent hinterland, which may not be covered by current laws restricting wetland development (Foster & Soluk, 2006), but is recognised by the IUCN Odonata Specialist Group as key to conserving odonates globally (Moore, 1997). These areas should certainly be included in any studies of odonates and their habitat, as different individuals may be present and may exhibit different behaviours.

Chapter 4: Estimating survival rates and population size in *Ischnura pumilio* populations

4.1. Introduction

Information about ecological parameters, such as survival rates and population sizes, can be crucial when considering species' conservation requirements. Differences in survival between groups within a population and the effect of external factors on survival rates can provide valuable information about how conservation management should be directed. The mark-release-recapture (MRR) methodology is widely used and well established for studying survival and estimating population sizes in wild populations.

Odonates are particularly suitable for study using MRR methods due to their ease of capture and marking, relatively short adult life span and restriction to aquatic habitat and surrounding areas. Many MRR studies of survival in odonates have been conducted and commonly reported higher survival rates in males than females (Banks & Thompson, 1985b; Fincke, 1986; Michiels & Dhondt, 1989). However, many of these studies did not consider differences in recapture probabilities between groups within the population, instead assuming an animal to be dead or dispersed if not resighted (Michiels & Dhondt, 1989; Anholt, 1991; Cordero, 1995). If groups differ in their probability of recapture (for example, due to behavioural differences between sexes), then differences in resighting rates cannot be directly attributed to differences in survival. It was often suggested that lower recapture probabilities in female odonates lead to reduced survival estimates (Garrison & Hafernik, 1981; Hinnekint, 1987; Cordero, 1994).

Modern techniques for modelling MRR data provide estimates of survival that account for differences in recapture probabilities. These methods are based on models developed by Cormack (1964), Jolly (1965) and Seber (1965), and more recently extended by Lebreton *et al.* (1992) to consider the

effects of time, age and categorical variables (e.g. sex), as well as external covariates such as weather. Using these methods, Cordero Rivera & Andrés (1999) found that survival rates did not differ between the sexes but that recapture rates were reduced in female *Ischnura pumilio*. The same was found by Anholt (1997) in a population of *Lestes disjunctus*, where the recapture rate of males was 2.5 times that of females. However, Anholt *et al.* (2001) captured a greater number of males than females in both *C. puella* and *I. elegans*, and MRR modelling revealed that the cause was a greater male recapture rate in the former but greater male survival in the latter.

Further advances to multistate MRR models (Arnason, 1973; Schwarz *et al.*, 1993; Lebreton & Pradel, 2002) allow individuals to move between a set of states during the intervals between capture occasions. Survival and recapture probabilities may be estimated separately for individuals in each state, along with the probability of transition between states. States may be defined as geographical sites or categorical individual covariates which change over time, such as state of maturity or parasite load. Recent studies have used these methods to address questions relating to dispersal (Fric & Konvička, 2002; Zimmerman *et al.*, 2007), and reproductive tradeoffs (Rivalan *et al.*, 2005; Townsend & Anderson, 2007).

Although many MRR studies of damselflies have been undertaken, this is the first to apply multistate modelling techniques, and is also the first to use these techniques to consider parasitism in damselflies or otherwise. This Chapter presents the results of both single and multistate models to analyse survival and recapture probabilities in two populations of *I. pumilio* in the south of England. The effects of sex, state of maturity, time, age, parasitism and weather were considered. The aim of this study was to provide information on the factors influencing survival rates in *I. pumilio* populations, in order to inform their conservation management. Information on recapture rates and their influencing factors will also be useful in determining effective survey techniques for future studies of the species.

Estimation of population size was the original focus of mark-recapture models (Schwarz & Seber, 1999), and although emphasis has moved to

estimation of survival rates in recent years, sophisticated and accurate population size estimates are still highly useful in conservation management. However, these estimates are rare for odonate populations. The transect walk method of monitoring population size originally developed for butterflies (Pollard, 1977), has also been used to monitor populations of dragonflies (Jenkins, 1986). This gives a measure of relative abundance which may reflect variation in absolute population size. The advantage of this method is that it can be carried out by virtually untrained field workers (e.g. members of the public) and as such has lead to the accumulation of some very large data sets (e.g. Butterfly Monitoring Scheme database). However, these methods are subject to bias due to heterogeneity of sighting (capture) probabilities as described above. Furthermore, in species which exist as adults only for one year or season such as odonates, total population size for that period might be of interest. Transect walks do not account for repeat sightings of individuals over occasions, considering only those visible at each individual occasion as independent events.

This Chapter presents Horvitz-Thompson estimates of daily and total population size and estimates of its variance produced using recapture rates from open population models (Horvitz & Thompson, 1952; Pollard, 1977; McDonald & Amstrup, 2001). The relationship between these estimates and those produced using other methods, for example simultaneously collected transect walk data, was also considered.

4.2. Methods

4.2.1. Study sites and populations

The two populations studied were those at Latchmoor Brook, New Forest and Great Wheal Seton, Cornwall and are described in Chapter 2. MRR data collection followed the methods described in Chapter 2. A total of 2052 individuals was captured, marked and released in 2005, and a further 12 individuals found dead were included in the analysis. In 2006, 252 individuals were captured and no dead individuals were recovered. Capture histories for each individual were constructed and analysis of survival and recapture probabilities (and transition probabilities for multistate analyses) was conducted using the program MSURGE (Choquet *et al.*, 2004). Capture histories from 2005 were gathered into two-day intervals due to the data being too sparse in single day interval format.

A multistate approach was used to model survival, recapture and transition probabilities for the 2005 data set. States were defined as state of maturity (immature or mature, as defined in Chapter 2) in order to investigate a potential difference in demographic rates before and after maturation. The same data were also used to consider an effect of parasite load on survival and recapture rates, again using a multistate approach. Only the first 20 two-day capture occasions were used for the mites analysis, as numbers of damselflies and mites observed began to decline past this time, and sparse data can cause problems identifying effects. Individuals with incomplete histories of mite load were also omitted, leaving 1737 individuals in this dataset.

Single state models were applied to the 2006 data set to estimate survival and recapture rates in the Cornish population and compare them to those for the New Forest population. This data set was not gathered into two-day capture occasions as this was not necessary. A subset of the 2006 data was used to test the effect of removing a leg on individual damselflies, as this practice is common during MRR studies for concurrent genetic analyses (e.g. Watts *et al.*, 2007a). A leg was taken from alternate individuals within each sex, until approximately 30 legs from each sex (33 male and 28

female) were obtained. The capture histories of these individuals, along with the first 30 from each sex captured without leg removal, formed the data set for the legs analysis.

4.2.2. Models and assumptions

The Jolly-Seber (JS) method (Jolly, 1965; Seber, 1965) uses summary statistics of numbers of marked and unmarked animals at each capture occasion to compute estimates of population size (N_t), survival rates (φ_t), recruitment numbers (B_t) and recapture probabilities (p_t). The following assumptions are typically listed for the JS model (Amstrup *et al.*, 2005).

1. Every animal alive in the population (marked and unmarked) at a given capture occasion (t) has an equal chance (p_t) of being captured (assumption of equal catchability).
2. Every animal in the population at a given capture occasion (t) has an equal chance of survival (φ_t) to the next capture occasion.
3. Marked animals do not lose their marks and marks are not overlooked.
4. Sampling periods are short relative to the intervals between them (recapture duration assumption).
5. Emigration is permanent.

The Cormack-Jolly-Seber model (Cormack, 1964; Jolly, 1965; Seber, 1965) requires information only on the recaptures of marked animals, and that these animals are representative of the population. This model is implemented using iterative techniques based on individual capture histories and uses probabilistic models for the events that give rise to those histories. Two primary parameters are used: the probability of a marked animal surviving and remaining in the population from time t to time t+1 (φ_t); and the probability that a marked animal in the population is captured at time t (p_t). The complete likelihood for the CJS model is the product of the probabilities for each capture history (Seber, 1982; Lebreton *et al.*, 1992). Maximum likelihood estimation allows estimates of the model parameters, φ_t and p_t, to be obtained. All parameters are time specific under this model. The first capture probability cannot be estimated and the final survival and

recapture probabilities cannot be separately estimated, only their product. The assumptions typically listed for the CJS model include those listed above for the JS model (assumptions 1 and 2 refer only to marked individuals in the case of the CJS) and one further assumption (Amstrup *et al.*, 2005):

6. The fate (recapture and survival probability) of each animal is independent of any other in the population.

Where group membership can change unpredictably through time, the CJS model can be generalised to include multiple states. These multistate models were initially developed to consider movement between geographical areas, but can also be used to consider states such as level of parasitism. The first of these models was the conditional Arnason-Schwarz (CAS) model (Arnason, 1973; Schwarz *et al.*, 1993) and produces estimates of the probability of transition (ψ) between groups conditional on survival, as well as survival (φ) and recapture (p) probabilities. In addition to variation through time, parameters in multistate models are also permitted to vary with current and previous state. Survival parameters are not permitted to vary with current state as transitions are assumed to happen instantaneously immediately before each capture interval, therefore survival cannot be influenced by state of arrival. CAS models do not allow recapture probabilities to depend on previous state, but a further generalisation to the Jolly-move (JMV) model (Brownie *et al.*, 1993) allows this dependency. In addition to the CJS assumptions listed above, multistate models make the following further assumptions:

7. State of recapture is recorded without error.
8. Movement probabilities are equal for all individuals in a given state at time t.
9. Movement and recapture probabilities do not depend on the past history of the animal (memoryless assumption).

Assumptions 1 and 2 are frequently violated in field studies, as recapture and survival rates often vary as a function of individual attributes. Substantial attention has been devoted to modelling this variation and

allowing survival (φ) and recapture (p) probabilities to be modelled as functions of time specific covariates such as environmental conditions, and to vary between groups within the population (e.g. Lebreton *et al.*, 1992). In addition, the full time dependence of the CJS model may be too general in some cases and survival or recapture probabilities which are constant over time more appropriate. The methodology of Lebreton *et al.* (1992), based on the CJS model but also applicable to multistate models, allows these problems to be accounted for by comparing reduced parameter models. The population may be partitioned into sub-populations or groups in order to consider the effect of variables such as sex and age (here defined as time since first capture). Time specific external covariates such as environmental variables may also be considered as potential effects on parameters. These additional effects may be applied to only survival probabilities, only recapture probabilities or both. Models may be specified so that two or more effects influencing a parameter do so in an interactive or additive manner.

4.2.3. Model selection

Model selection is performed by first choosing a fully-parameterised starting model which accounts for the major structural features of the data, then reducing the parameterisation of subsequent models toward a more parsimonious model. The CAS model was used as the starting model for the 2005 maturity and mites analyses. This was preferred to the JMV as dependence of capture probabilities on state of maturity or mite load at the previous capture occasion did not seem particularly biologically meaningful. Also, reduced parameterisation of recapture specification allows greater power to consider effects on survival. The single state analyses of the 2006 data used the CJS model. The best model in a candidate set is identified using the Akaike Information Criterion (AIC), which selects the most parsimonious model according to the formula $AIC = 2k - 2\ln(L)$, where k is the number of parameters and L is the likelihood function. This methodology attempts to find the model that best explains the data with a minimum number of parameters, which is identified by the lowest AIC value.

Due to the vast number of possible parameter combinations, model selection was performed in stages. As transition parameters were of least interest, these were dropped from the model first, keeping full parameterisation for survival and recapture. Next the recapture parameters were reduced in the same way, keeping full parameterisation for survival. At the end of each stage, the model with the lowest AIC value was used for parameter reduction in the next stage. For example, if ψ_f was selected as the best model for transition parameters, for the next stage of model selection considering recapture parameters, ψ_f was used in the model specifications. Deleted effects from model specifications with similar AIC values to the "best" model at the end of each stage were added to the best model at the end of the following stage, to confirm that the hypothesised effects were still unsupported (Lebreton *et al.*, 1992). For example, the second and third best models for transition parameters were reconsidered once the best model for recapture parameters had been found. An *a priori* set of models was defined to include and exclude each of the considered effects in an interactive manner. Additive effects were also included where they seemed biologically plausible. A degree of *post hoc* model simplification was then undertaken, to ensure the selected effects should be included in the best model. A list of all considered models for each analysis appears in Appendix 2.

Normalised Akaike weights (w_i) were used to consider the relative plausibility of the best models given the data (Burnham & Anderson, 1998). These weights were calculated as

$$w = \frac{\exp(-\Delta AIC/2)}{\sum\{\exp(-\Delta AIC/2)\}}$$

Akaike weights not only show which model is best, but also by how much in relation to the next best model. They allow statements to be made about the results without resorting to arbitrary significance levels, and can be thought of as the probability that a model is correct or the proportion of support for it in the data (Cooch & White, 2006).

4.2.4. Goodness of fit tests

Model selection based on AIC requires that at least one model in the set considered fits the data adequately. Otherwise, overparameterised models may be incorrectly selected due to inflated model deviance values. This can lead to incorrect biological conclusions and bias in parameter estimates (Pradel *et al.*, 2005). The umbrella or most general model must therefore be shown to fit the data using goodness of fit (GOF) tests. Begon (1983) showed that less than 11% of studies using the Jolly-Seber method quantitatively addressed or discussed the assumptions of the model. Since then several general methods for testing GOF have been developed (Pradel *et al.*, 2005). Modern GOF procedures allow a global test of fit to the CJS model (Pollock *et al.*, 1985) which incorporates specialised tests for transience (Pradel *et al.*, 1997a) and trap dependence (Pradel, 1993). These tests and the multistate equivalents are implemented in the program UCARE, allowing the user to formally test the fit of the relevant model to the data.

Departures from underlying assumptions indicated by GOF tests can be of two types: structural failure of the model or extra-binomial variation (Lebreton *et al.*, 1992). Structural failure due to transience (Brownie & Robson, 1983) or trap dependence (Pradel, 1993; Gimenez *et al.*, 2003) can be accounted for by modifying the umbrella model to incorporate these effects. Once structural problems are resolved, extra-binomial variation can be caused by heterogeneity of true survival and recapture rates and/or failure of the 'iii assumption' (independence of fates and identity of rates between individuals; a combination of assumptions 1, 2 and 6). This can be summarised by a variance inflation factor ĉ, which indicates the amount of residual variation present in the data (Lebreton *et al.*, 1992). There are several methods for calculation of ĉ (Cooch & White, 2006), but this study uses that recommended by Lebreton *et al.* (1992), which is the ratio χ^2/df (pooled chi squared statistics and degrees of freedom from GOF tests). This is modified when structural modifications to the umbrella model are made, by subtracting the values for χ^2 and degrees of freedom from the relevant component of the GOF test. For example if transience is indicated, ĉ is

calculated as (overall χ^2 – test 3G.SR χ^2)/(overall df - test 3G.SR df) (Choquet *et al.*, 2005b).

GOF tests were implemented using the program UCARE 2.2 (Choquet *et al.*, 2005b). UCARE tests the fit of the JMV model to the data for multistate and the CJS model to single state data. A further test of the suitability of the CAS model as the umbrella model for a multistate data set can be performed using a likelihood ratio test (LRT) of the JMV vs. the CAS models (Pradel *et al.*, 2003). A likelihood ratio test (LRT) is used to test for a significant difference in fit between two nested models. Provided the reduced parameter model is satisfactory, the difference in deviance between the two models is distributed as χ^2, with degrees of freedom equal to the difference in number of parameters (Cooch & White, 2006). Therefore, a non-significant result indicates that the two models fit the data equally well, and the simpler of the two should be preferred based on parsimony. As the CAS model is nested within the JMV, the CAS is a suitable umbrella provided the LRT test is not significant.

4.2.5. Model specification

In all analyses it was expected that survival and recapture probabilities could be dependent on sex (*s*) and/or time (*t*). Survival probabilities were also considered potentially to depend on age. Multistate analyses also allowed survival, recapture and transition probabilities to be modelled with an effect of state. State of departure is symbolised "*f*" (from) and state of arrival "*to*"; transition and recapture parameters can be modelled with an effect of both or either, but only an effect of state of departure on survival can be considered. For the 2005 analysis, states were defined as immature and mature (as defined in Chapter 2). As an individual cannot become immature once mature, the probability of that transition was set to zero when specifying the models. For the mites analysis, states were defined as mite load categories. Individuals were assigned to one of three states: no mites, low mite load (1 to 4 mites), or high mite load (5 or more mites, maximum 33). Where the damselfly was captured on both days of the capture occasion the maximum mite number during the two-day period was

used. The effect of various daily weather variables (maximum temperature, minimum temperature, rainfall, solar radiation, wind speed, cloud cover) were included in the analysis as covariates of time, along with a negative linear trend as a potential effect of age.

4.2.6. Population size estimates

Estimates of recapture probabilities from the best models in each analysis were used to calculate estimates of population size using a Horvitz-Thompson type estimator (Horvitz & Thompson, 1952; McDonald & Amstrup, 2001). The estimated population size (N_t) at each time (t) is calculated as:

$$N_t = n_t / p_t$$

where n_t is the number of individuals recorded during each capture period, and p_t is the recapture probability for that period. Variances for the estimates were calculated using the following formula (McDonald & Amstrup, 2001):

$$\hat{\text{var}}(\hat{N}_t) = \sum_{i=1}^{n} \left[\frac{n_{it}(1-\hat{p}_{it})}{\hat{p}_{it}^2} + \frac{n_{it}\hat{\sigma}^2_{\hat{p}it}}{\hat{p}_{it}^3} + \frac{n_{it}(1-\hat{p}_{it})\hat{\sigma}^2_{\hat{p}it}}{\hat{p}_{it}^4} \right]$$

Approximate 95% confidence interval for the population size estimates were calculated using the Wald method (Evans *et al.*, 1996).

$$\hat{N} \pm 1.96\sqrt{\text{var}(\hat{N})}$$

Where the calculated value for the lower limit was less than numbers seen, the lower limit was amended to equal numbers seen (Huggins, 1989). Confidence intervals calculated using the Wald method perform well for estimates based on a large sample size when compared to bootstrapped or profile likelihood methods which are far more computationally intense (Evans *et al.*, 1996). Therefore, the intervals estimated for population size estimates in the central portion of the study period are likely to be good. However, they may be more unreliable for those estimates at the start and end of the study period, and are specifically likely to underestimate the

upper limit (Evans *et al.*, 1996). However, given the time requirements of a more sophisticated estimate such as profile likelihood, the confidence intervals estimated using the Wald method were judged to be adequate for these analyses.

In order to obtain an estimate of total population size, this method was repeated using newly captured individuals only. This requires that unmarked individuals have the same capture rate as marked individuals, as no recapture estimate is made for the first capture occasion by the models used. This assumption was justified as no difference in behaviour or visibility was noted between the two groups. The estimated number of new individuals was calculated as above and summed to obtain a total estimate. Variances for daily estimates were also summed to give an overall variance estimate (M. Spencer, pers. com.).

Although some individuals were omitted from the data set for the modelling procedure due to missing data, all were used in the calculation of population size as an accurate value for numbers seen is required. Individuals missing data on number of mites were assigned to the 0 mites category as this was the most common (86.9% of all captures).

For comparison, daily population size was also estimated using the Jolly-Seber (JS) method as implemented in the program Simply Tagging (Pisces Conservation, 2003). The two series of estimates were compared to numbers of unique individuals seen and minimum numbers alive (MNA) at each occasion. MNA is the sum of all individuals known to be alive during each capture period. An individual is known to be alive if it was captured during a capture session, or before and after that capture session. In order to compare the various methods, *I. pumilio* data was combined with *Coenagrion mercuriale* data from Chapter 5, and one pair of daily estimates from each site (individual *C. mercuriale* sites considered separately) to form two datasets. The first contained maximum HT estimates for each site and the corresponding daily number of captures. The second contained every population size estimate (number of captures, HT, JS and MNA) for a randomly selected day at each site.

On 18 consecutive days in 2006 a transect walk was conducted at Great Wheal Seton, Cornwall, by walking an established route through the habitat and counting the number of individuals observed using a method adapted from Pollard & Yates (1994). Although partly determined by the structure of the site and the walkways within it, the route was designed to cover the range of habitat types within the site from good to poor. Walks were conducted between 11am and 3pm in order to limit diurnal and temperature related effects on observed numbers (Harker & Shreeve, 2008). Males and females were counted separately as they are easy to distinguish in this species. These counts were compared to their corresponding daily HT estimates. Statistical analyses were performed in R 2.4.0 (R Development Core Team, 2005). A map of the transect walk route is shown in Appendix 1.

4.3. Results

4.3.1. 2005 analysis

Initial GOF tests revealed that the JMV model $\psi_{f*to*t}\ \varphi_{f*t}\ p_{f*to*t}$ was strongly rejected for males (χ^2 = 148.216, P = 0.001, df = 98) but supported for females (χ^2 = 122.582, P = 0.992, df = 163). Examination of the component tests revealed that males failed test 3G.SR (χ^2 = 58.391, P = 0.001, df = 21), which considers the null hypothesis that there is no difference in the probability of being reencountered between new and old individuals. This initial structural failure was accounted for by including a two-age-class effect on survival (Brownie & Robson, 1983) to account for a transience (individuals passing through the population) or marking effect. The two-age-class effect allows survival estimates to vary between the first interval and all subsequent intervals. As the umbrella model to be used in this analysis was the CAS model, a further likelihood ratio test of JMV *vs.* CAS was performed. The LRT test revealed no difference in fit between the two umbrella models (χ^2 = 24.617, P = 1, df = 104) so model simplification proceeded from the CAS.

GOF tests also indicated that the data violated the memoryless assumption (that transition between states does not depend on previous states) as test WBWA was also significant. This is due to the definition of states as immature and mature, transition between which can clearly have only one direction. Departures from the assumptions of multistate models of this type may be dealt with by use of memory models (Brownie & Robson, 1983) which are beyond the scope of this thesis, or by applying a variance inflation factor (Pradel *et al.*, 2005; Choquet *et al.*, 2005b). The variance inflation factor, ĉ, was calculated as (overall χ^2 – test 3G.SR χ^2) / (overall df - test 3G.SR df) (Choquet *et al.*, 2005b). A value of 0.888 was calculated for ĉ, indicating underdispersion in the data. When data are overdispersed, indicated by a ĉ value greater than 1, this can be accounted for by modifying AIC calculations using ĉ to give a $QAIC_c$ value. However, when ĉ <1 the default value of 1 is retained as this suggests no lack of fit, therefore modification of AIC is unnecessary (Amstrup *et al.*, 2005; Cooch & White,

2006). Underdispersion can be an artefact of sparse data (Schwarz, 2002) and this is supported by the problems encountered modelling the data in single day interval format.

Table 4.1. The best 10 models based on AIC values for the 2005 maturity analysis. Number of parameters (NP), model deviance, Akaike information criterion (AIC), AIC differences (ΔAIC) and AIC weights. Subscripts – a = age (*NL* indicates negative linear effect, 2 indicates two-age-class effect), f = state of maturity on previous occasion, to = state of maturity at current occasion, s = sex, t = time.

Model	NP	Dev	AIC	Δ AIC	AIC weight
$\Psi_{f*s}\ \varphi_{[a\ NL]+t+f*s}\ p_{t+s}$	59	12027.557	12145.557	0.0000	0.7053
$\Psi_{f*s}\ \varphi_{[a\ NL]+t+f}\ p_{t+s}$	57	12033.707	12147.707	2.150	0.2407
$\Psi_{f*s}\ \varphi_{[a\ NL]+t}\ p_{t+s}$	56	12040.848	12152.848	5.141	0.1831
$\Psi_{f}\ \varphi_{[a\ NL]+t+f}\ p_{t+s}$	56	12054.981	12166.981	19.274	0.0002
$\Psi_{f}\ \varphi_{[a\ NL]+t+f}\ p_{to\ [t+s]}$	81	12005.647	12167.647	19.940	0.0001
$\Psi_{f}\ \varphi_{[a\ NL]+t}\ p_{t+s}$	55	12063.141	12173.141	25.434	0.0000
$\Psi_{f*s}\ \varphi_{a2+t+f}\ p_{to*[t+s]}$	83	12007.443	12173.443	25.736	0.0000
$\Psi_{f}\ \varphi_{a+t+f}\ p_{to*[t+s]}$	100	11979.931	12179.931	32.224	0.0000
$\Psi_{f}\ \varphi_{a+f+t+s}\ p_{to*[t+s]}$	101	11977.988	12179.988	32.281	0.0000
$\Psi_{f}\ \varphi_{a+t+s}\ p_{to*[t+s]}$	99	11982.047	12180.047	32.340	0.0000

Values of deviance, AIC, difference in AIC, AIC weight and number of parameters for the best 10 models are listed in Table 4.1. In excess of 90 models were tested altogether and are listed in Appendix 2. Using AIC as the tool for model selection, the model $\psi_{f*s}\ \varphi_{[a\ NL]+t+f*s}\ p_{t+s}$ was the best explaining the data. Model subscripts in this analysis were defined as: a - age, f - state of maturity on previous occasion, to - state of maturity at current occasion, s – sex and t - time. The best model indicates that survival rates varied with time and that maturity and age had an additive effect. The effect of age (defined here as time since first capture) was best described with a negative linear trend, and the effect of maturity differed with sex. Recapture rates also varied through time with an additive effect of sex. Probability of transition between states depended on sex, and previous state of maturity also featured in the model specification as transition can only occur if an individual is immature.

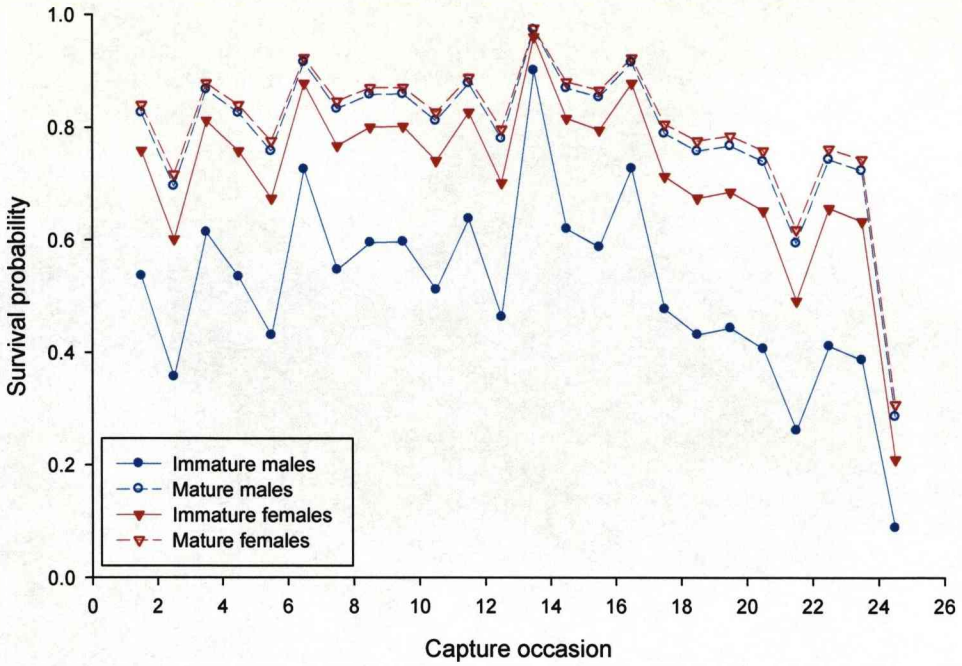


Figure 4.1. Maximum likelihood estimates of survival probability for 2005 maturity data. Rates for newly captured individuals are shown. Lines connecting values for the same sex and state are intended for orientation only. Estimates were calculated by MSURGE using the model $\Psi_{f*s}\ \varphi_{[a\ NL]+t+f*s}\ p_{t+s}$.

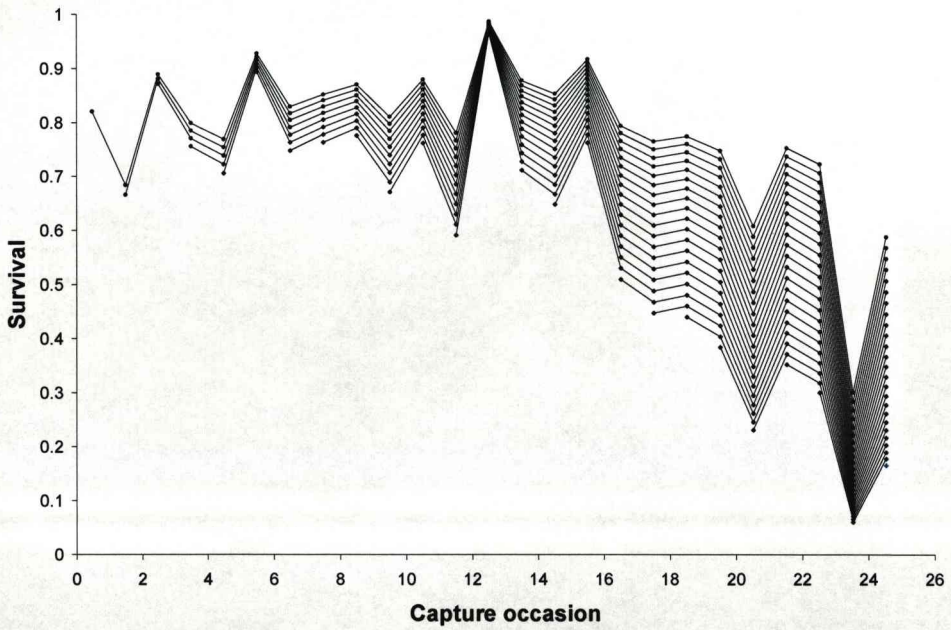


Figure 4.2. Maximum likelihood estimates of survival probability for 2005 maturity data. Points at each capture occasion represent new individuals (age class 1) at the top, sequentially through age classes to the maximum age possible at that time at the bottom (e.g. age class 10 at occasion 10). Rates for mature individuals are shown. Lines connecting values for the same age class are intended for orientation only. Estimates were calculated by MSURGE using the model $\Psi_{f*s}\ \varphi_{[a\ NL]+t+f*s}\ p_{t+s}$.

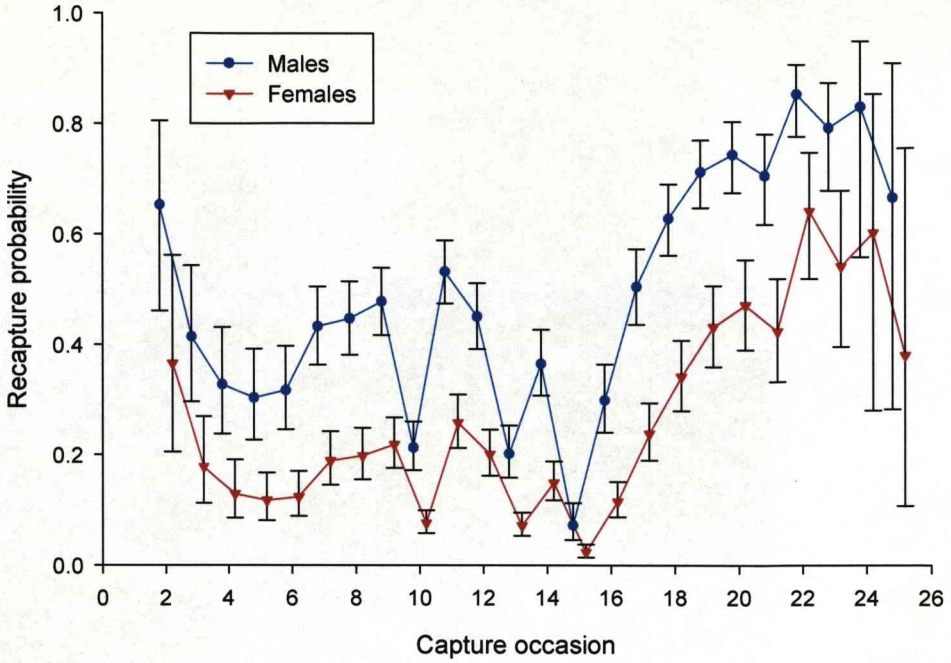


Figure 4.3. Maximum likelihood estimates of recapture probability for 2005 maturity data. Lines connecting values for the same sex are intended for orientation only. Estimates and error bars representing 95% confidence intervals were calculated by MSURGE using the model $\Psi_f \varphi_{[a\ NL]+t+f} p_{t+s}$.

The AIC weights show that the best model had almost three times as much support in the data as the next model $\psi_{f*s}\ \varphi_{[a\ NL]+t+f}\ p_{t+s}$. The best model was therefore used to obtain parameter estimates, which are shown in Figs. 4.1 to 4.3. Final recapture and survival estimates are confounded when both are time dependent as they cannot be estimated separately (Lebreton *et al.*, 1992; Gimenez *et al.*, 2003). Therefore, calculated values can not be interpreted in the same way as all other estimates and as such have been omitted from figures throughout where a fully time dependent model is shown.

Based on the model $\psi_{f*s}\ \varphi_{[a\ NL]+t+f*g}\ p_{t+s}$, survival in mature males and females was almost identical, but immature males had a greatly reduced probability of survival compared to both immature females and mature individuals (Fig. 4.1). Survival rates were lower for immature individuals than mature, and decreased linearly (on a logit scale) with age, here defined as time since first capture (Fig. 4.2). Error bars are omitted from some figures in this chapter to aid interpretation, but the level of error in the estimates as calculated by MSURGE was generally large. Recapture rates

were higher in males (Fig. 4.3). Both survival and recapture rates exhibited temporal variation, but none of the weather variables considered was a significant influencing factor on either. The estimated probability of transition from immature to mature in any two-day period was 1 for males and 0.36 for females.

The calculated two-daily survival rates from this study were converted to estimated daily survival rates by taking the square root (Cook *et al.*, 1967), assuming that survival is approximately constant across two-day intervals. The average survival rate for mature male *I. pumilio* was 0.77 and for females 0.79, which correspond to daily rates of 0.88 and 0.89 respectively. For immature individuals mean two daily and daily survival rates for males were 0.48 and 0.70, and for females 0.70 and 0.84 respectively.

4.3.2. Mites analysis

The *I. pumilio* population of Latchmoor Brook was parasitised by mites of the genus *Hydryphantes.* The larvae of these mites encounter adult damselfly hosts while walking on the water's surface or nearby vegetation (Smith, 1988) and can therefore attach at emergence or at a later time such as when damselflies visit the water for mating or oviposition. Of the 1737 individuals included in this analysis, most had no mites at first capture (93.7% of males and 78.3% of females). Females were more intensely parasitised than males; 18.5% had between 1 and 4 mites at first capture and 3.2% had 5 or more, compared with 6.0% and 0.3% respectively in males. These percentages were almost identical when all captures and recaptures were considered. Mature females were also parasitised more frequently (1-4 mites 21.5%, 5+ mites 4.8%) than immature females (1-4 mites 7.6%, 5+ mites 0.0%), suggesting mites continued to attach throughout a female's life. The distribution of mites on newly captured individuals was non random (Chi squared test against Poisson distribution, males: $\chi^2 = 1324.89$, $df = 6$, $P < 0.001$; females: $\chi^2 = 15697.33$, $df = 11$, $P < 0.001$). The variance to mean ratio of mite numbers recorded at each recapture indicated that the distribution of mites in males (8.02) and females (4.95) was aggregated in

some individuals. Parasite load increased during the season and declined towards the end (Fig. 4.4).

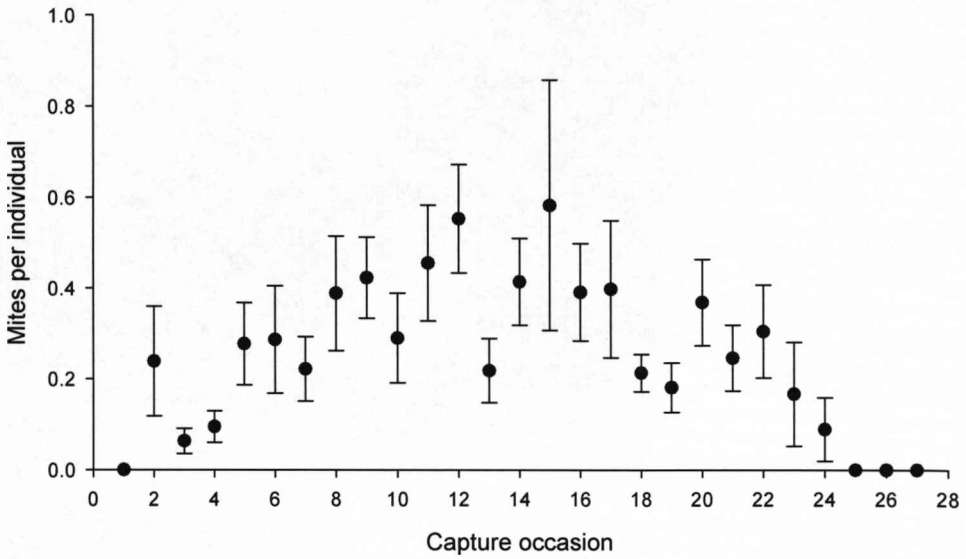


Figure 4.4. Mean number of mites recorded per individual during the 2005 study period. Error bars represent 1 standard error.

The CAS model (ψ_{f*to*t} φ_{f*t} p_{to*t}) was supported overall for males and females. However, closer examination of the individual tests again revealed some evidence for structural failure due to a transience or marking effect on males, indicated by near significance of the test 3G.SR (χ^2 = 45.283, P = 0.060, df = 32). This was accounted for as above, by starting model selection from a two-age-class model, and removing the values of χ^2 and degrees of freedom for test 3G.SR from the calculation of ĉ. This cautious approach was taken because an age effect was identified in the same data in the 2005 analysis. Females failed the M.LTEC test (χ^2 = 14.708, P = 0.040, df = 7), which tests for a difference in the expected time and state of reencounter between those individuals encountered and not encountered at a given occasion. This can be accounted for with the variance inflation factor ĉ (Choquet *et al.*, 2005b). An overall value of 0.574 was calculated for ĉ, so again no modification was made to the default value of 1 in MSURGE. A likelihood ratio test of JMV *vs.* CAS showed that the two models fit the data equally well (χ^2 = 49.468, P = 0.780, df = 58), therefore the CAS model was used as the umbrella model.

Values of deviance, AIC, difference in AIC, AIC weight and number of parameters for the best 10 models tested are listed in Table 4.2. In total, 115 models were tested and are listed in Appendix 2. The model $\psi_{f*to+s}\ \varphi_{a2*s+t+f}\ p_{s*to+t}$ was the best explaining the data based on AIC. Model subscripts in this analysis were defined as: *a* - age, *f* – mite load on previous occasion, *to* – mite load on current occasion, *s* – sex and *t* - time. The AIC weights show that the second model had almost twice as much support in the data. The only difference between the models was an effect of parasitism on survival; therefore an LRT test was performed to assess the significance of this effect. The result of the test was non significant although close to the 0.05 boundary (χ^2 = 5.2559, P = 0.072, df = 2) therefore the simpler model excluding the effect of mites should be preferred based on parsimony. However, as the test was so close to significance, the model including parasite level was used to obtain parameter estimates in order to examine the effect on parameters. Examination of the parameter estimates indicated that transition and recapture parameters were unaffected by the inclusion of a mite effect on survival, and that the effect of other variables on survival parameters was also unaffected.

Table 4.2. The best 10 models based on AIC values for the 2005 mites analysis. Number of parameters (NP), model deviance, Akaike information criterion (AIC), AIC differences (ΔAIC) and AIC weights. Subscripts – *a* = age (2 indicates two-age-class effect), *f* = mite load on previous occasion, *to* = mite load on current occasion, *s* = sex, *t* = time.

Model	NP	Dev	AIC	ΔAIC	AIC weight
$\psi_{f*to+s}\ \varphi_{a2*s+t+f}\ p_{s*to+t}$	55	8294.847	8404.847	0.000	0.6069
$\psi_{f*to+s}\ \varphi_{a2*s+t}\ p_{s*to+t}$	53	8300.103	8406.103	1.256	0.3239
$\psi_{f*to+s}\ \varphi_{a2+t+f}\ p_{s*to+t}$	53	8305.872	8411.872	7.025	0.0181
$\psi_{f*to}\ \varphi_{a2*s+t}\ p_{s*to+t}$	52	8307.888	8411.888	7.041	0.0180
$\psi_{f*to+s}\ \varphi_{a2+t}\ p_{s*to+t}$	51	8310.149	8412.149	7.302	0.0158
$\psi_{f*to+s}\ \varphi_{a2+f+t+s}\ p_{s*to+t}$	54	8305.723	8413.723	8.876	0.0072
$\psi_{f*to+s}\ \varphi_{t+f}\ p_{s*to+t}$	52	8311.669	8415.669	10.822	0.0027
$\psi_{f+to}\ \varphi_{a2*s+t}\ p_{s*to+t}$	51	8313.682	8415.682	10.834	0.0027
$\psi_{f*to+s}\ \varphi_{a2*f+t}\ p_{s*to+t}$	55	8305.97	8415.97	11.123	0.0023
$\psi_{f*to+s}\ \varphi_{s+t+f}\ p_{s*to+t}$	53	8311.666	8417.666	12.819	0.0010

The model $\psi_{f*to+s}\ \varphi_{a2*s+t+f}\ p_{s*to+t}$ indicated that survival rates differed between new and recaptured individuals and between the sexes and that these effects were interactive (Fig. 4.5). Survival was greater for newly marked males than those later recaptured, but survival of newly marked

females was reduced. Survival in recaptured individuals was marginally lower in males than females. Survival rates also decreased with increasing mite load (Fig. 4.6). The average survival rate for males was 0.67 and for females 0.71, which correspond to daily rates of 0.82 and 0.84 respectively. These values were converted into average life expectancies using the method recommended in Cook *et al.* (1967). The calculated life expectancy for males was 5.04 and for females 4.02 days. These values are considerably lower than estimates of mean life span calculated from the original data as time between first and last capture (males = 8.23; females = 8.06 days). This may due to some exceptionally long capture histories biasing the mean in the latter estimate. These calculations were not performed for the previous analysis, as incorporating immature survival rates would require weighting mean survival by number of days alive in each state which is somewhat circular.

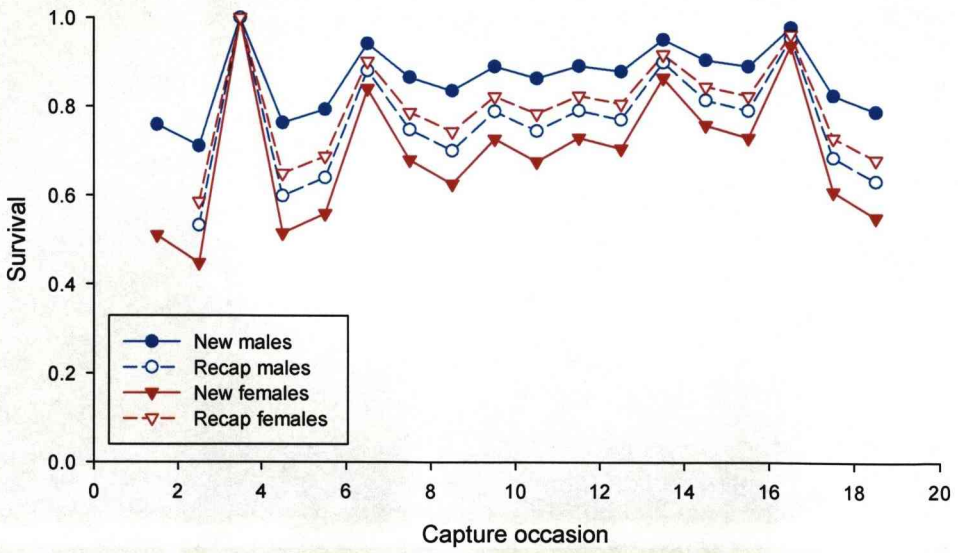


Figure 4.5. Maximum likelihood estimates of survival probability for 2005 mites data showing the difference in rate between new and recaptured individuals and between males and females. Estimates for individuals with no mites are shown. Lines connecting values for the same sex and state are intended for orientation only. Estimates were calculated by MSURGE using the model $\Psi_{f*to+s}\ \varphi_{a2*s+t+f}\ p_{s*to+t.}$

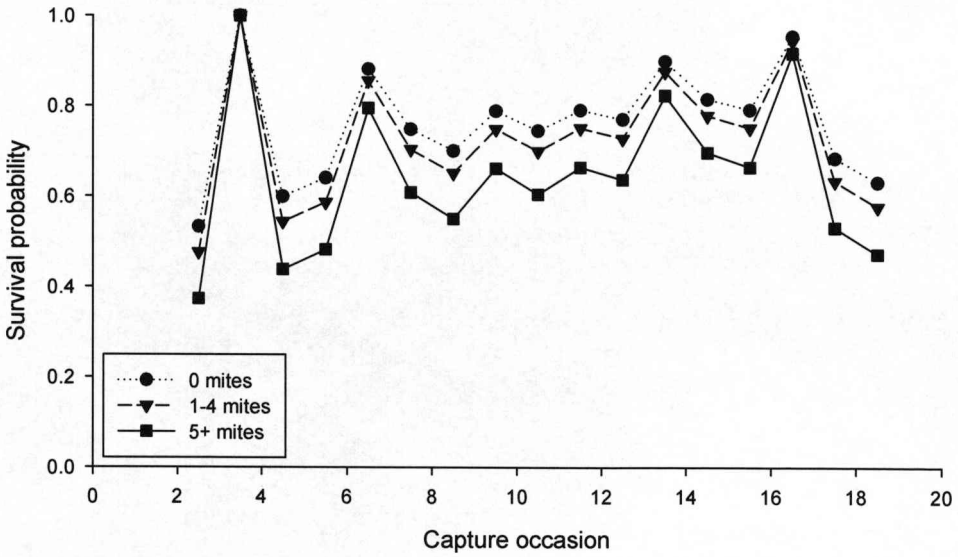


Figure 4.6. Maximum likelihood estimates of survival probability for 2005 mites data showing the effect of mite load on males after the first capture interval (females show same pattern). Lines connecting values for the same state are intended for orientation only. Estimates were calculated by MSURGE using the model $\Psi_{f*to+s}\ \varphi_{a2*s+t+f}\ p_{s*to+t}$.

Recapture rates varied interactively with level of parasitism and sex, and also varied through time (Fig. 4.7). Individuals with a low mite load had the highest recapture probability in both sexes. Probability of transition between levels of parasitism was dependent on both previous and current mite load, and also varied with sex (Table 4.3). Males were most likely to remain at the same level of parasitism, regardless of the preceding state, although transition from a low level to no mites was also common (0.395). Females were also highly likely to remain in the same state overall, but those with a low level of parasites were more likely to lose them all (0.542) than retain a low level (0.367).

Table 4.3. Probability of transition between levels of mite load calculated by program MSURGE using the model $\psi_{f*to+s}\ \varphi_{a2*s+t+f}\ p_{s*to+t}$.

Males	**Current**				**Females**	**Current**		
Previous	**0**	**1-4**	**5+**		**Previous**	**0**	**1-4**	**5+**
0	0.962	0.037	0.000		**0**	0.927	0.072	0.001
1-4	0.395	0.538	0.066		**1-4**	0.542	0.367	0.091
5+	0.032	0.000	0.968		**5+**	0.062	0.000	0.938

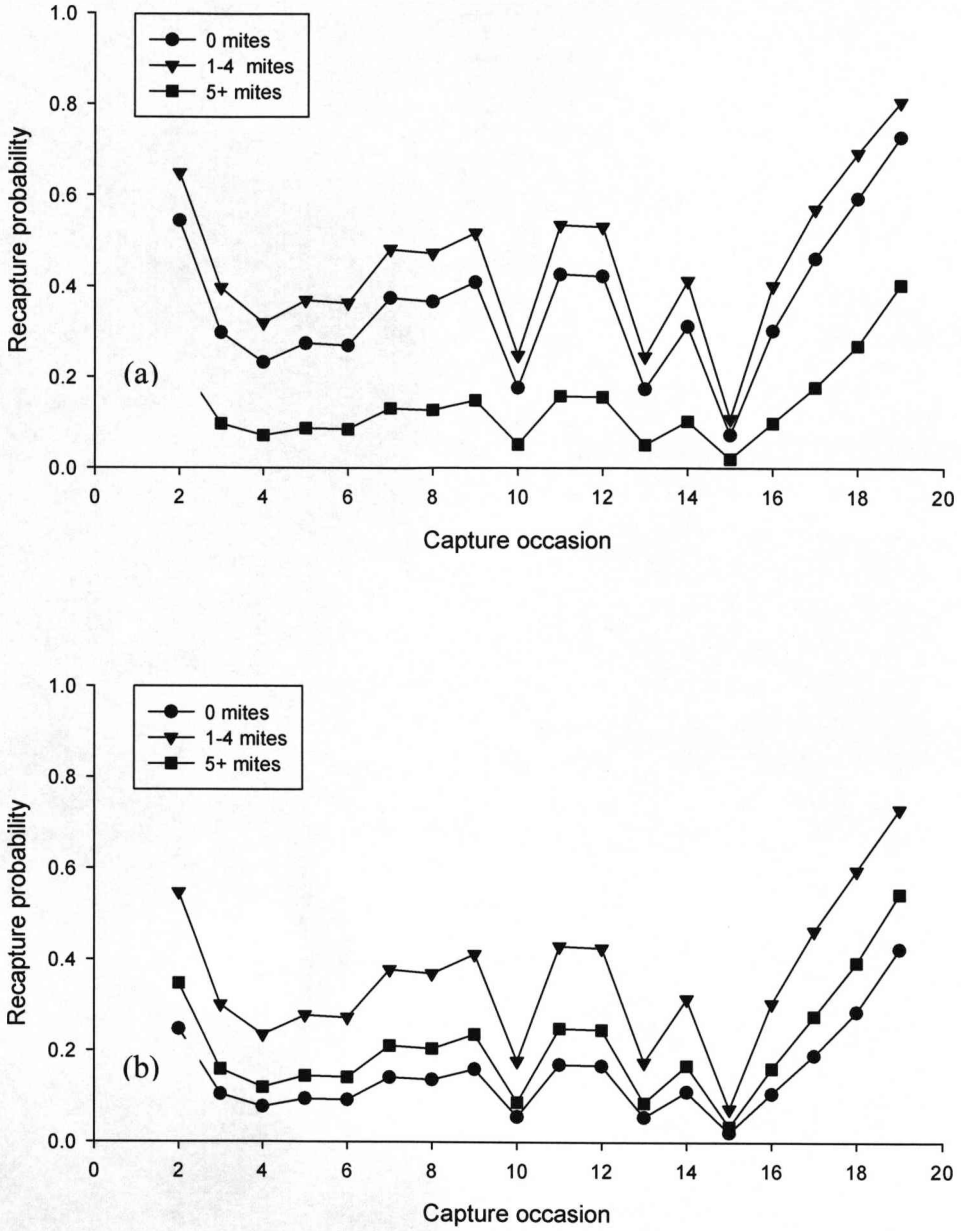


Figure 4.7. Maximum likelihood estimates of recapture probability for 2005 mites data showing the effect of mite load on males (a) and females (b). Lines connecting values for the same state are intended for orientation only. Estimates were calculated by MSURGE using the model $\Psi_{f*to+s}\ \varphi_{a2*s+t+f}\ p_{s*to+t}$.

4.3.3. 2006 analysis

Initial GOF tests again suggested a significant transience effect in males, as although test 3.SR for males was non-significant overall (χ^2 = 21.607, P = 0.486, df = 22) sub-components of this test were significant. Therefore, a two-age-class effect on survival was included, as this allowed comparison with the 2005 analysis. A value of 0.708 was calculated for ĉ, indicating underdispersion in the data. The default value of 1 was retained for the model selection process as explained above.

Table 4.4. The best 10 models based on AIC values for the 2006 analysis. Number of parameters (NP), model deviance, Akaike information criterion (AIC), AIC Differences (ΔAIC) and AIC weights. Subscripts – *a2* = two-age-class effect, *s* = sex, *t* = time (*MX* and *MN* indicate maximum and minimum temperature constraints respectively).

Model	NP	Dev	AIC	ΔAIC	AIC weight
$\varphi_{a2}\, p_{t+s}$	28	2015.532	2071.532	0.000	0.178
$\varphi_{a2+t[MX]}\, p_{t+s}$	29	2013.709	2071.709	0.177	0.163
$\varphi_{a2}\, p_{t}$	27	2018.137	2072.137	0.605	0.132
$\varphi_{a2+t[MN]}\, p_{t+s}$	29	2014.231	2072.231	0.699	0.126
$\varphi_{a2+t[MX]}\, p_{t}$	28	2016.403	2072.403	0.871	0.115
$\varphi_{a2+s}\, p_{t+s}$	29	2015.487	2073.487	1.955	0.067
$\varphi_{a2+t[MX]+s}\, p_{t+s}$	30	2013.565	2073.565	2.034	0.065
$\varphi_{a2*s}\, p_{t+s}$	30	2013.661	2073.661	2.129	0.062
$\varphi_{a2+s}\, p_{t}$	28	2017.896	2073.896	2.365	0.055
$\varphi_{a2*s}\, p_{t}$	29	2016.691	2074.691	3.160	0.037

Values of deviance, AIC, difference in AIC, AIC weight and number of parameters for the best 10 models tested are listed in Table 4.4. In total, 38 models were tested and are listed in Appendix 2. The model $\varphi_{a2}\, p_{t+s}$ was the best explaining the data based on AIC. Model subscripts in this analysis were defined as: *a* - age, *s* – sex and *t* - time. This model indicates that survival was reduced in the first interval following capture compared to all subsequent intervals. Recapture rates varied through time with an additive effect of sex (Fig. 4.8).

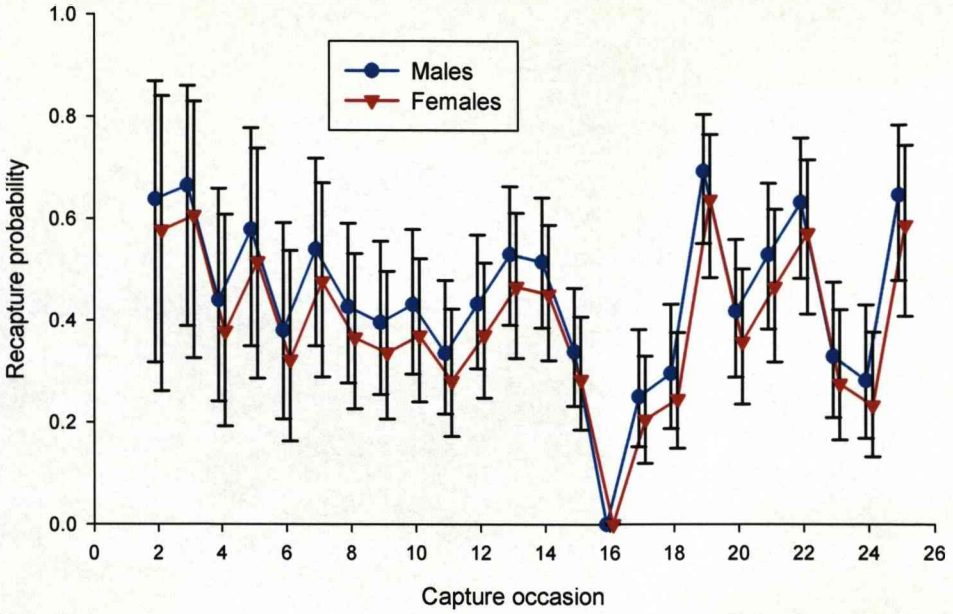


Figure 4.8. Maximum likelihood estimates of recapture probability for 2006 data. Lines connecting values for the same sex are intended for orientation only. Estimates and error bars representing 95% confidence intervals were calculated by MSURGE using the model $\Phi_{a2}\, p_{t+s}$.

The AIC weights show that this model had a similar amount of support in the data as the next model $\varphi_{a2+t[MX]}\, p_{t+s}$, which includes an effect of maximum temperature on survival. However, an analysis of deviance (ANODEV) revealed no significant effect of temperature ($F_{(1,23)} = 1.30$, P = NS). An ANODEV is appropriate for testing the significance of cohort-level, as opposed to individual-level covariates, and is based on an F ratio (Skalski *et al.*, 1993; Johannesen & Ims, 1996). The test partitions models' deviances in the way that ANOVA partitions models' sums of squares, and produces F ratios that are interpreted in the same way. In this case the numerator quantifies the amount of variation attributable to temperature, and the denominator quantifies the residual temporal variation. The results of this analysis are presented in Table 4.5.

Table 4.5. Analysis of deviance testing for an effect of maximum temperature on survival rates *I. pumilio* (2006). Corrected total deviance and degrees of freedom (df) represent the difference in respective values between the models $\varphi_{a2+t}\, p_{t+s}$ and $\varphi_{a2}\, p_{t+s}$. Total covariate deviance and df represent the difference in values between models $\varphi_{a2}\, p_{t+s}$ and $\varphi_{a2+t[MX]}\, p_{t+s}$. Mean deviance (MD) is obtained by dividing deviance by its df, and F is the ratio (total covariate MD) / (error MD)

Source	df	Deviance	Mean Deviance	*F*	P
Corrected total	24	34.113			
Total covariate	1	1.823	1.823	1.298	NS
Error	23	32.290	1.404		

The third model in the list also has 75% as much support in the data as the best model, indicating that the effect of sex on recapture rate may not be significant. An LRT test to compare the models $\varphi_{a2}\, p_{t+s}$ and $\varphi_{a2}\, p_t$ indicated no significant difference in fit between them ($\chi^2 = 2.605$, P = 0.107, df = 1) therefore the simpler model is preferred. This suggests that the effect of sex on recapture parameters is small and non-significant. This is illustrated in Fig. 4.8, which shows recapture parameter estimates from the best model, where values for males and females at a given interval are visibly similar.

Daily survival rates from the best model were 0.71 for individuals in their first interval following capture and 0.88 for any subsequent interval. Average life expectancy was calculated using the latter estimate as 7.82 days, and was greater than mean life span calculated from the original data (6.42 days).

4.3.4. Legs analysis

GOF tests revealed no departure from the assumptions of the model $\varphi_t\, p_t$ in this subset of the 2006 data. A ĉ of 0.249 was calculated, indicating severe underdispersion of the data, which may be due to the small data set. The default value of 1 was again retained in the model selection process.

Values of deviance, AIC, difference in AIC, AIC weight and number of parameters for the best 10 models tested are listed in Table 4.6. In total, 23 models were tested and are listed with the above values in Appendix 2. Model subscripts in this analysis were defined as: l - leg removal, s – sex and t - time. The model $\varphi_i\, p_i$ was the best explaining the data based on AIC. This indicates that in this subset of data, no variation in daily survival or recapture rates was detected. The daily rate of survival from the best model was 0.836, and the recapture rate 0.414. This model had over twice as much support in the data as the model containing an effect of leg removal on survival ($\varphi_l\, p_i$). An LRT test indicated no significant difference between the best model, $\varphi_i\, p_i$, and the model, $\varphi_i\, p_l$, ($\chi^2 = 0.0516$, df = 1, P = 0.820), confirming that effect of leg removal on survival is not significant.

Table 4.6. The best 10 models based on AIC values for the legs analysis. Number of parameters (NP), model deviance, Akaike information criterion (AIC), AIC differences (ΔAIC) and AIC weights. Subscripts – s = sex, l = leg removal, "." = no effects.

Model	NP	Deviance	AIC	ΔAIC	AIC weight
$\varphi_. p_.$	2	1066.589	1070.589	0.000	0.327
$\varphi_. p_s$	3	1066.425	1072.425	1.837	0.131
$\varphi_. p_l$	3	1066.466	1072.466	1.877	0.128
$\varphi_l p_.$	3	1066.537	1072.537	1.948	0.124
$\varphi_s p_.$	3	1066.575	1072.575	1.986	0.121
$\varphi_l p_s$	4	1066.374	1074.374	3.785	0.049
$\varphi_s p_s$	4	1066.423	1074.423	3.834	0.048
$\varphi_l p_l$	4	1066.439	1074.439	3.850	0.048
$\varphi_{s*l} p_.$	5	1066.515	1076.515	5.926	0.017
$\varphi_. p_t$	25	1028.979	1078.979	8.390	0.005

4.3.5. Population size estimates

Parameter estimates from the best model in the mites analysis were used to calculate population sizes for 2005, as these were deemed more reliable due to less GOF issues. Although these estimates do not cover the end of the study period, it is unlikely to affect population sizes as most individuals would have emerged by capture occasion 20 (14th and 15th July 2005). Only 60 new individuals were captured after this time and all but two were mature. Estimates are shown separately for males and females in Fig. 4.9 so that sex ratio may be discussed. Estimates from the 2005 maturity analysis are also presented for comparison with all other estimates in Fig. 4.10, and follow a similar pattern to the mites model estimates although generally smaller. The maximum estimated number occurred at capture occasion 10 which represents the 24th and 25th of June 2005. An estimated 1508 (± 259) individuals were present in the population during this period, comprising 633 (± 108) males and 876 (± 236) females. Wald 95% confidence intervals are given in parentheses. The overall population size for the season calculated using the Horvitz-Thompson estimator with new individuals as described above was 8723 (± 470) comprising 3064 (± 166) males and 5659 (± 440) females.

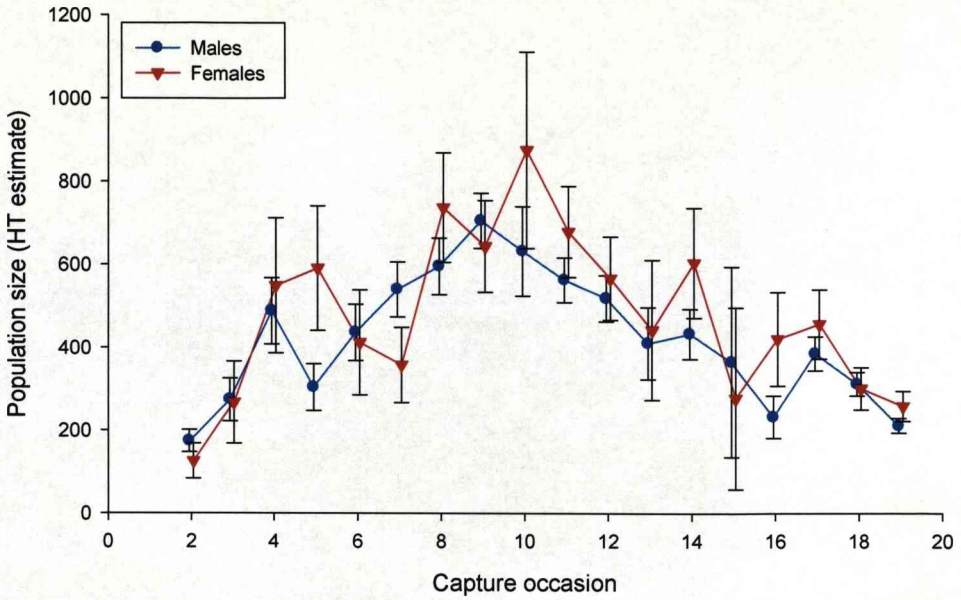


Figure 4.9. Number of adult males and females in the Latchmoor population (2005) estimated using the Horvitz-Thompson (HT) method with parameter estimates from the model $\Psi_{f*to+s}\ F_{a2*s+t+f}\ p_{s*to+t}$ and the mites data set. Lines connecting values for the same sex are intended for orientation only. Errors bars represent 95% Wald confidence intervals.

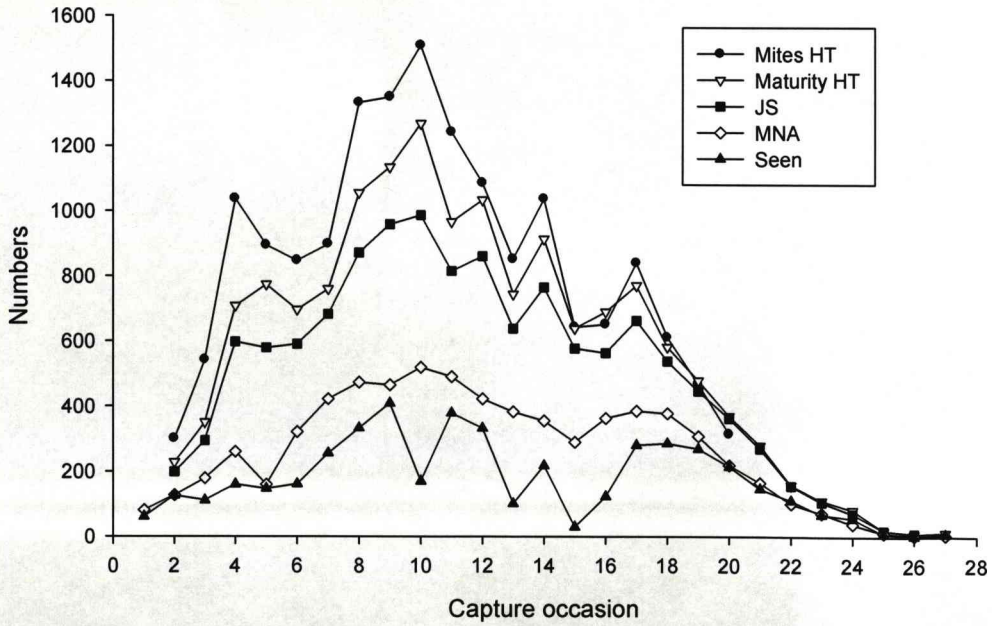


Figure 4.10. Estimates of the size of the Latchmoor Brook *I. pumilio* population in 2005, calculated using different methods. HT = Horvitz-Thompson, MNA = minimum number alive, JS = Jolly-Seber. Lines connecting values for the same method are intended for orientation only.

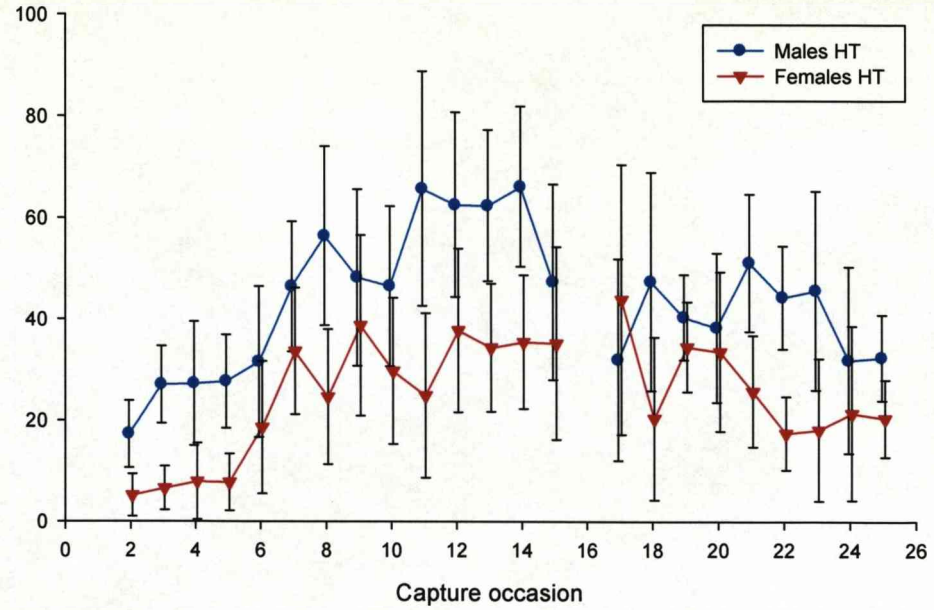


Figure 4.11. Number of adult males and females in the Cornish population (2006) estimated using the Horvitz-Thompson (HT) method with parameter estimates from the model φ_{a2} p_{t+s}. Lines connecting values for the same sex are intended for orientation only. Errors bars represent 95% Wald confidence intervals.

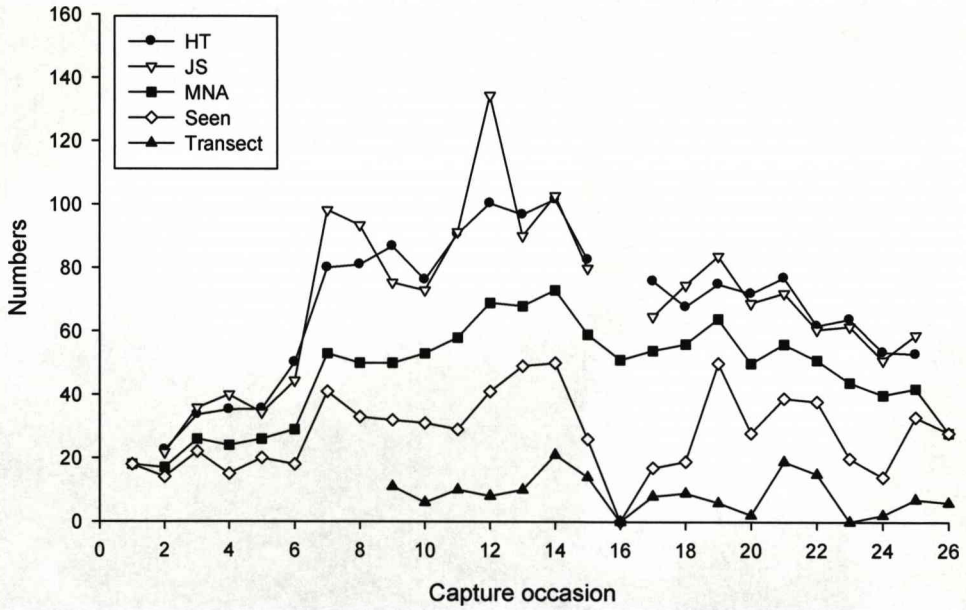


Figure 4.12. Estimates of the size of the Great Wheal Seton *I. pumilio* population in 2006, calculated using different methods. HT = Horvitz-Thompson, MNA = minimum number alive, JS = Jolly-Seber and Transect walk counts. Lines connecting values for the same method are intended for orientation only.

Table 4.7. Total numbers of *I. pumilio* in both study years. Observed values (Obs: total numbers marked) and Horvitz-Thompson (HT) estimates are presented with their 95% Wald confidence intervals.

Year	**2005**		**2006**	
	Obs	HT	Obs	HT
Males	1138	3064 ± 166	162	315 ± 40
Females	914	5659 ± 440	90	212 ± 38
Total	2052	8723 ± 470	252	527 ± 55

The 2006 population did not exhibit a definite peak in numbers (estimated using the HT method), but the highest estimate of 102 (± 21) individuals occurred on day 14 comprising 66 (± 16) males and 36 (± 13) females (Fig. 4.11). Where no individuals were recorded on a capture occasion, no HT estimate or variance could be calculated, and no data point appears on the graph. The overall estimated population size for the study period was 527 (± 55), comprising 315 (± 40) males and 212 (± 38) females. Observed and estimated overall population sizes for the *I. pumilio* populations studied in both years are summarised in Table 4.7 and daily estimates are presented in Figs. 4.10 and 4.12.

A total of 18 transect walks was carried out on consecutive days during the 2006 study period (occasions 9 to 26; Fig. 4.12). The mean percentage of the population (as estimated by HT) recorded by transect walks was 26.8% (s.e. = 10.4% calculated as $\sqrt{(p(1-p)/n)}$ where p = mean percentage; Crawley, 2005). Daily percentages ranged from 0 (when no individuals were observed despite their known presence) and 53%. Both methods of estimation placed maximum abundance on 17th June 2006 and also identified a second small peak in abundance on 21st June 2006. No negative relationship between the proportion of the population recorded by transect walk and HT estimates was found (r = 0.210, P = 0.402), indicating that transect walks were equally as reliable as an index regardless of density.

There was a significant positive relationship between transect counts and HT estimates of population size (r_s = 0.532, P = 0.023). As these data were all taken from the same population, the data pairs were not independent and so the residuals of the linear model were examined for temporal autocorrelation (Keller-McNulty & McNulty, 1987; Crawley, 2007). A linear regression of the data was performed in order to examine residuals. The distribution of both Pollard walk counts and HT estimates was approximately normal as were the residuals of the linear model. A positive relationship between the two estimates was again identified (r = 0.600, P = 0.008). A plot of residuals against time revealed no trend as did an autocorrelation plot of the residuals; therefore the assumption of independence was upheld.

The relationship between maximum daily HT estimates and number of *I. pumilio* and *C. mercuriale* recorded using MRR counts was also positive, but non-significant (r = 0.443, P = 0.233). However, the datum for *C. mercuriale* at the Lower Itchen Complex (LIC) was an outlier. This large HT estimate was from a period of bad weather, during which capture numbers varied greatly across consecutive days and sites. Consequently, this may be an overestimate due to problems modelling days with few captures. Removing this datum from the analysis resulted in a significant positive relationship (r = 0.955, P = 0.0002). The slope of the linear regression model was 3.48 and the intercept 281. Although the explanatory power of this model is very high (r^2 = 0.913), it should be treated with caution for predictive purposes due to the exclusion of a significant outlier. A significant positive relationship was also found between MRR counts and HT population size estimates based on randomly selected data pairs from each site (r = 0.784, P = 0.012).

Jolly-Seber estimates of population size correlated most strongly with HT estimates (r = 0.999, P < 0.00001) and are visually similar across days and locations (Figs. 4.10 and 4.12). The slope of the linear model was 0.967 and the intercept 95.26 indicating that JS estimates consistently underestimate HT population size by approximately 95 individuals.

4.4 Discussion

4.4.1. Assumptions

Initial departure from the assumptions of umbrella models in all data sets due to a transience or marking effect was accounted for by fitting a two-age-class model. The 2005 maturity data set also failed the memoryless assumption; that movement and recapture probabilities do not depend on the past history of the animal. This occurred because the states were defined as immature and mature, and transition between these states can clearly only occur in one direction. The suggested alternative to using a memory model (which is beyond the scope of this analysis) in these circumstances is to modify ĉ and proceed with caution (Choquet *et al.*, 2005b). However, the calculated value of ĉ for this analysis was less than 1 and therefore was not modified for model selection and parameter estimation. Therefore, although all the relevant advice was followed as far as possible, the parameter estimates and effects selected may be biased by the departure from assumptions and should be treated with caution. Of the effects considered in both the maturity and mites analyses, the same were selected in both final models. This suggests the effect of violating the memoryless assumption was minimal as the analyses use the same data.

Heterogeneity of survival or recapture rates and/or non-independence of individuals fates (assumptions 1, 2 and 6) can cause overdispersion (Lebreton *et al.*, 1992), indicated by a value of ĉ greater than 1. As all the calculated values of ĉ were less than 1, it is unlikely that these assumptions were severely violated. Sparse data can cause underdispersion and may have contributed to that observed here. Individuals were marked with a spot of paint on the thorax in addition to the unique ID code in order to ensure that marks were not lost or overlooked (assumption 3). On the rare occasion (0.8% of captures in 2005, 1.5% in 2006) that the wing carrying the ID was lost or the number obscured, this was known due to the paint mark, and these individuals were omitted from analyses. Very little long range movement was recorded in these populations (Chapter 2), therefore it seems unlikely that there was a significant amount of emigration. However, as the

proposed mechanism of dispersal between populations in this species is carriage on wind currents, it seems highly likely that any emigration that did take place would have been permanent (assumption 5).

The recapture duration assumption (4) is violated in this study as long capture periods (9 hours) relative to the interval between them (15 hours) were necessary to obtain sufficiently large sample sizes. Furthermore, gathering the data into two-day capture intervals significantly increased the capture period to interval ratio. However, O'Brien *et al.* (2005) recommend this approach, as they found increased precision of parameter estimates but no increased bias when the recapture duration assumption was violated in favour of a larger sample size in simulation and empirical data. Furthermore, Lebreton *et al.* (1992) state that the effect of violating this assumption is minimised when using models more complex than the JS.

4.4.2. 2005 analysis

Survival rates varied through time, declining towards the end of the season (Fig. 4.1). Stochastic variation in survival rate was probably due to environmental conditions, although no direct effect of any of the tested weather variables was found. However, weather variables such as temperature, solar radiation and rainfall may have complex interactive effects on survival which are not detectable individually. Rain may reduce survival in some species (e.g. Córdoba-Aguilar, 1994) but it is unlikely that rainfall during the study was sufficiently heavy to have an effect. Decreased survival at the end of the season may be expected, as it approaches the end of the main flight season for *I. pumilio* (Smallshire & Swash, 2004). Almost all individuals would have emerged by mid July due to very good weather, resulting in an aging population thereafter with increasing mortality rates.

The age of an individual (days since first capture) also influenced survival; rates were higher in mature individuals compared to immature and decreased linearly with age within those stages. Although survival in mature males and females was almost identical (Fig. 4.1), immature males had a greatly reduced probability of survival compared to both immature females

and mature individuals. Maturation in males is much more rapid than in females; taking approximately one day following the teneral stage (Cham, 1993). Definition of the immature period is somewhat subjective and can be based on colouration or the state of the gonads but no general standard exists (Corbet, 1999). Although tenerals were excluded from this study, males may have been more vulnerable during the stage defined here as immature, due to incomplete hardening of the cuticle and development of flight. As such these males may have suffered higher mortality from predators, intra-specific aggression and handling. Female maturation takes longer, so many females captured during this period were past the teneral stage and consequently more robust to the previously mentioned causes of damage. This effect may also be due to a problem with sparse data as only 4% of new males were immature compared to 31% of females.

Many studies of damselflies have concluded that males live longer than females, but often suggested that lower female recapture probabilities may have lead to apparent reduced survival (Garrison & Hafernik, 1981; Hinnekint, 1987; Cordero, 1994). Models which estimate survival and recapture rates separately allow some resolution of this problem (Anholt, 1997). Cordero Rivera & Andrés (1999) found that survival rates did not differ between the sexes but that recapture rates were reduced in female *I. pumilio,* which concurs with results for mature individuals in this study. The same effect was found in a population of *Lestes disjunctus* where the recapture rate of males was 2.5 times that of females (Anholt, 1997), in *Pyrrhosoma nymphula* (Bennett & Mill, 1995), and also in a butterfly, *Parnassius clodius*, (Auckland *et al.*, 2004).

Cordero (1994) found reduced survival in immature males in four damselfly species including *I. pumilio*. Male *Ceriagrion tenellum* suffered greater mortality during the pre-reproductive period than females, and the same pattern occurred in laboratory populations, although mature males had slightly higher survival (Andres & Cordero Rivera, 2001). In a study of *Ischnura elegans,* Anholt *et al.* (2001) concluded that males suffered greater mortality whilst immature, as the predicted sex ratio calculated using adult survival and recapture rates was more male biased than observed numbers.

Indirect estimates of survival in other species have suggested that females have higher mortality rates than males during maturation (Anholt, 1991; 1997), which might be expected due to greater energetic demands for growth and egg maturation, but others have found no such effect (Córdoba-Aguilar, 1993; Bennett & Mill, 1995; Stoks, 2001b).

Sex had a significant effect on recapture probability (Fig. 4.3), with males twice as likely to be recaptured as females on average. This is probably due to differential behaviour between the sexes and differences in colouration. Males are present at the water every day that weather conditions are suitable. Females however, spend most of their time away from water in the surrounding vegetation, feeding and avoiding harassment from males (Banks & Thompson, 1987; Anholt, 1992; Stoks, 2001a, b). Although these areas were searched regularly, females mostly remain hidden within vegetation unless disturbed or hunting. Male *I. pumilio* are also much more brightly coloured than mature females, which may have influenced the recapture rate, although immature females are highly visible due to their bright orange colouration.

Recapture rates also varied stochastically through time, again probably due to the influence of weather which influences odonates location within a habitat (Foster & Soluk, 2006; Chapter 3) and therefore their ease of capture. Recapture rates were highest at the beginning and end of the season, as numbers were low but capture effort remained the same. There was a general decline in recapture rates in the busiest part of the season (c. weeks 4 to 6) when numbers seen were at their highest (Figs. 4.9 & 4.10) and exceeded the maximum number it was possible to process in a day. A two-way ANOVA was performed to assess the effect of week and sex on the log-transformed time to next capture (Table 4.8). Only the first movement of individuals captured more than once was used, and the few first movements recorded after week 6, these were omitted. The effects of both sex ($F_{(1,1190)} = 30.40$, $P < 0.01$) and week ($F_{(5,1190)} = 20.40$, $P < 0.01$) were significant, but a non-significant interaction term was removed from the model ($F_{(5,1190)} = 0.56$). The model was further simplified by combining weeks with no significant difference between them (Crawley, 2005).

Therefore, the final model had 3 categories: early season comprising weeks 1, 2 and 3; week 4; and late season comprising weeks 5 and 6. The interval between captures increased in the middle of the study when numbers were highest, and was significantly larger in the fourth week than any other (Fig. 4.13). This confirms that individuals known to be present in the population were missed more frequently in the early to middle part of the study period, causing recapture rates to be lower.

Table 4.8. Linear model output of a two-way ANOVA considering effect of sex and week on $\log_{10}$ time to next capture, a non-significant interaction term was removed. Stars indicate significant differences (* P<0.05, *** P<0.001) between each term and the initial term (week 1/female). Differences between other pairs were deemed significant if greater than the sum of their standard error (SE) terms.

	Estimate	SE	*t*	P
Intercept	1.10678	0.07686	14.399	***
Week 2	0.12426	0.08331	1.492	
Week 3	0.15844	0.07803	2.03	*
Week 4	0.40092	0.08988	4.46	***
Week 5	-0.32309	0.09249	-3.493	***
Week 6	-0.38727	0.09766	-3.965	***
Sex (male)	-0.27897	0.05055	-5.519	***

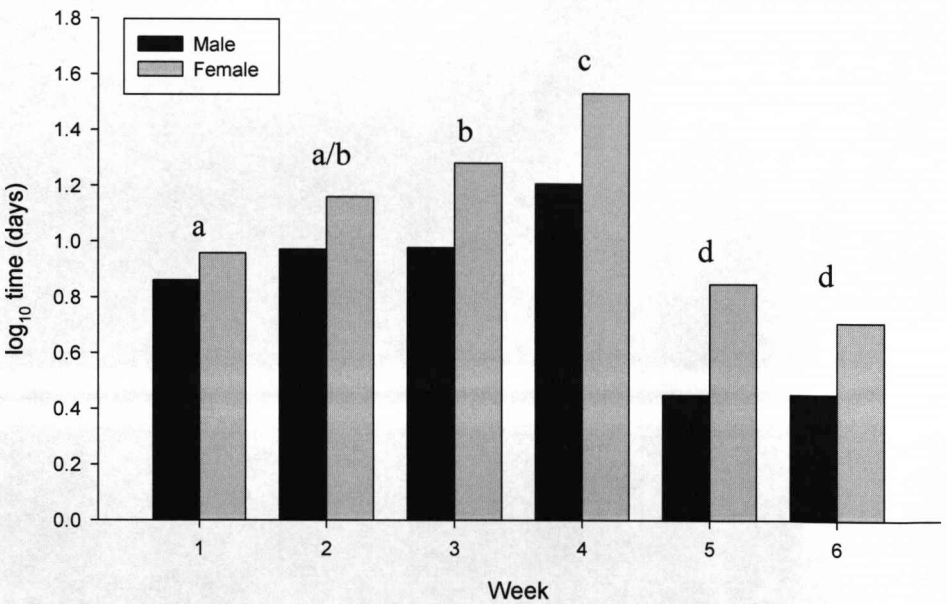


Figure 4.13. The effect of study week on time (days) to next capture in 2005. Males and females differ significantly, as do weeks with different letters above the respective bars (2-way ANOVA of $\log_{10}$ times – Sex: $F_{(1,1198)} = 32.29$, $P < 0.01$, Week: $F_{(2,1198)} = 47.78$, $P < 0.01$).

Higher recapture rates in males have been reported in many damselfly studies (e.g. Parr & Parr, 1972; Waage, 1972; Garrison, 1978; Van Noordwijk, 1978; Bennett & Mill, 1995; Anholt, 1997; Andres & Cordero Rivera, 2001) although Duffy (1994) found higher recapture probabilities for females of *L. disjunctus,* and Robinson (1983) found recapture rates to be highest in mature female *Ischnura posita.* In studies where observations were restricted to the water and water's edge, increased recapture rates in males may be expected as males and females use this part of their habitat differently (see Chapter 3). This remained a problem in the present study, albeit to a lesser degree, where the surrounding area was included.

Probability of transition between states of maturity was dependent on sex (as well as previous state, as transition was only possible in one direction). Males spend only a day or two as immatures, whereas females can take up to 12 days to reach maturity (Cham, 1993) and this was reflected by the estimates of transition to maturity over a two-day period of 1 for males and 0.36 for females.

4.4.3. 2005 Mites analysis

There was an effect of sex on survival in newly marked individuals but very little on recaptured individuals. New males had greater survival than recaptured individuals whereas female survival rates were reduced in comparison. In the previous analysis, survival was found to decline with age since first capture across all ages, whereas here a two-age-class effect was sufficient to describe the age effect. This is probably due to the omission of the last 7 capture occasions for the mites analysis, as this excludes age classes above 40 days (20 capture occasions) in which survival rates were much reduced (all below 0.4). These low survival estimates for older age classes would have favoured a negative linear effect of age, whereas when removed a two-age-class effect is sufficient. The longest recorded lifespan was 31 days in this study, but can reach 57 days in the laboratory (Cordero, 1994).

Male survival was lower in subsequent capture intervals than the first, as was the case for both sexes in the previous analysis. However, female survival in the mites analysis was lower during the first interval. This may be due to the effect of maturity on female survival found in the previous analysis. As so few immature males were recorded, they will contribute little to the calculation of survival rates in the mites analysis where maturity is not considered. However, 31% of newly captured females were immature which would have influenced survival rates in this analysis, potentially resulting in reduced survival rates for new females, as they were more likely to be immature and so have reduced survival. Cordero (1994) found that mortality following marking was low in captive *I. pumilio,* which is supported by the findings reported here to some degree. Survival in the first interval was increased in males, so it can be concluded that males are not negatively affected by marking. Females may be negatively affected to some extent or may exhibit reduced survival during the first interval simply due to reduced survival during the maturation stage. Following the first interval, females had a slightly higher survival rate than males. This has also been found in laboratory populations of this species (Cordero, 1994) and is likely to be due to increased flight activity and damage from intrasex aggression in males.

Survival was negatively affected by mite load (Fig. 4.6); rates were decreased by 4.9% on average in individuals with 1 to 4 mites, and by 15.0% when 5 or more mites were present, compared to individuals with no mites. However, the inclusion of an effect of mite load on survival in the final model was not fully supported (see results). The suggested effect is discussed here, but further work will be required before the effect of mites on survival in *I. pumilio* can be confidently established. Parasitism by water mites is common in damselflies, and has been found to negatively affect survivorship in some odonates (Robinson *et al.*, 1983) and insects in general (Smith, 1988 and refs therein). Mites are thought to drain considerable amounts of body fluids from their host (Åbro, 1990). As such they are likely to deplete nutrients and energy reserves and alter internal water balance (Smith, 1988), all of which may have an effect on mortality. Parasitised individuals are also likely to increase foraging behaviour, due to a need to

replace nutrients drained by mites. This will further increase energetic requirements, and also lead to increased predation risk. Mites have been found to reduce flight ability in damselflies (Reinhardt, 1990) and in insects in general (Smith, 1988). However, movement was found to be slightly increased in parasitised individuals in this study (see Chapter 2). Increased parasite-induced-dispersal could confound a mark recapture study as permanent emigration and death are indistinguishable. However, the increased movement detected in parasitised individuals in this species occurred only over short, within site distances, and as such is more likely to genuinely reduce survival due to increased energy demands.

Parasitism by water mites has been found to negatively affect survival in various insect groups (see Smith, 1988 for a review) but the effects on damselfly species are varied (Forbes *et al.*, 2004 and refs therein). Andrés & Cordero (1998) found no effect of intensity of parasitism on survivorship in *Ceriagrion tenellum*, but 98% of the population considered was parasitised, preventing comparison between individuals with and without mites. Robinson (1983) found a negative effect of mites on mature female survival in *Ischnura posita,* but was unable to detect an effect on males. A stronger effect of mites on female survival in laboratory populations of *Coenagrion puella* has also been reported, and parasitised females with relatively low mass at emergence suffered greater mortality in field cages (Braune & Rolff, 2001). A higher level of parasitism was associated with decreased longevity in food-deprived male *Enallagma ebrium* and reduced fecundity in females (Forbes & Baker, 1991).

Most studies have considered the effects of *Arrenurus* spp. mites on odonate hosts and Léonard *et al.* (1999) studied *Limnochares americana* parasitizing *E. ebrium*. To the author's knowledge this is the first study to consider the effects of *Hydryphantes* mites on an odonate. Although the effects of different genera of mites on a damselfly once attached are likely to be very similar, as they all attach to the host by piercing the exoskeleton and engorge on the internal tissues and haemolymph of the host. However, the modes of attachment to the host vary between genera. *Arrenurus* spp. mites attach immediately after emergence of damselfly larvae from the larval skin,

and remain anchored by their chelicerae until fully engorged. *Limnochares* and *Hydryphantes* spp. are free living and can transfer freely from vegetation or the water's surface to a host, and *vice versa*, allowing the number of mites per individual to vary over successive visits to the water (Smith, 1988; Fig. 4.14). Léonard *et al.* (1999) also reported higher prevalence and intensity of mite parasitism in older individuals. Their experimental study of *L. americana* on *E. ebrium* revealed that survival was only affected by a high level of parasites, and that a low level had no effect. However, they note that survival may be reduced indirectly by a sub-lethal level of parasites due to reduced anti-predator responses, which may contribute to the overall effect on survival in this study.

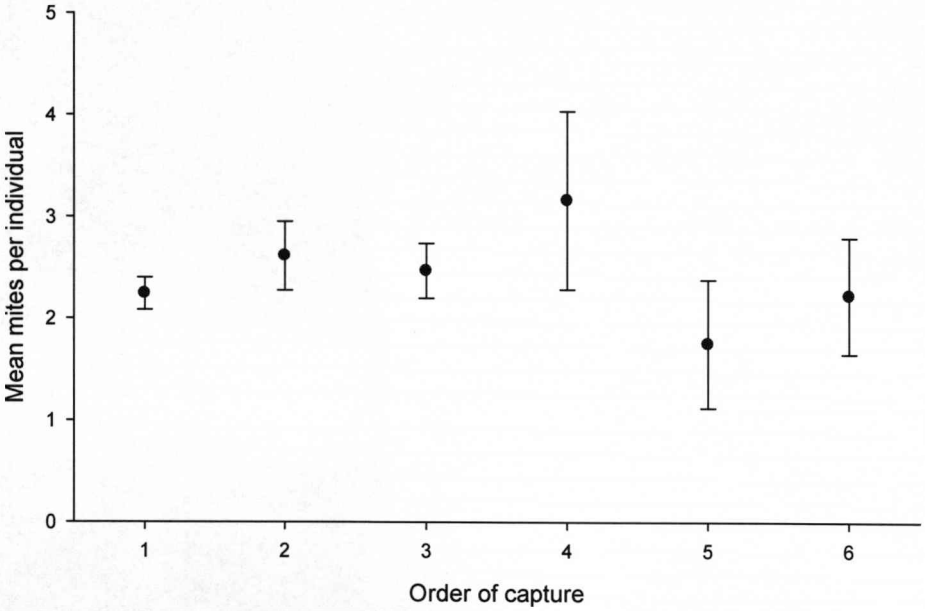


Figure 4.14. Mean number of mites recorded per individual on successive captures during the 2005 study period. Only individuals with at least one mite are shown as mite distribution was found to be aggregated. Error bars represent one standard error.

A very similar effect of sex and time on recapture rates to that found in the previous analysis was revealed here, with an additional effect of mite load (Fig. 4.7). Individuals with a low level of parasites had the highest recapture probability in both sexes. However, unparasitised females were less likely to be recaptured than females with mites, regardless of the intensity of infestation; whereas unparasitised males were almost as likely to be recaptured as those with low mite load, but with a high mite load were least

likely to be recaptured. This difference between the sexes may be due to behavioural differences. Males are more likely to be captured than females in general, and their recapture rate was only greatly reduced by a high level of parasitism. This may be caused by severe depletion of energy and resources causing a reduction in activity. A low level of mites however, may deplete resources enough to necessitate extra foraging behaviour, which would increase their visibility and therefore capture rate, but not enough to diminish activity.

A low level of mites may cause increased foraging behaviour leading to increased recapture rates in females also. However, females with a high level of mites were more likely to be recaptured than their unparasitised counterparts, suggesting that females are more resistant to the effects of heavy mite loads than males, and are able to increase their foraging behaviour to a level above that necessary in the absence of parasites. This may be due to their larger size compared to males. Alternatively, as mite distribution was aggregated, it may be that females visiting the water more frequently to mate are subject to more opportunities for mite attachment, and also for capture. Conversely, those females visiting the water less frequently will have lower recapture rates and are less likely to acquire mites. In contrast to this, Robinson (1983) found that mite-free, *Ischnura posita* females had a greater recapture rate than mite infested females.

Mites were found to be aggregated on certain individuals, and to parasitize females more than males. The apparent preference for females is surprising as generally females spend less time at the water. However, behaviour when at the water differs considerably between the sexes and this may account for the increased parasitism of females. Males are often actively searching this area for mates, whereas females visit only for mating and oviposition. It is likely to be during oviposition when a female is most susceptible to attachment of mites, as she must make regular contact with the water's surface and emergent vegetation for two hours or more in some cases (Fox & Jones, 1991). Male *I. pumilio* do not accompany females during oviposition and are therefore not subject to this prolonged exposure. A preference of mites for females over males has been found in previous

studies of odonate species (Andres & Cordero, 1998; Forbes *et al.*, 2004), but in territorial species such as *Leucorrhinia frigida* the pattern may be reversed as males spend large amounts of time defending perches which are frequented by mite larvae (Smith & Cook, 1991). However, in the laboratory male *L. frigida* are less susceptible to mite parasitism than females, suggesting a sex bias in grooming efficiency may exist, which is masked by different exposure rates in the field (Léonard *et al.*, 1999).

Transition rate estimates indicated that individuals were likely to remain at the same level of parasitism, regardless of the preceding state, although females with a low level were more likely to lose all their mites, and this was also quite likely in males. This indicates that females were parasitised for a shorter time than males. Recapture rate was increased in parasitised females, whereas male recapture rates were severely negatively affected by high levels (Fig. 4.7). If mites can fully engorge and be ready to detach faster on a female host, this might explain the preference for female hosts observed. If more body fluids are drained over a shorter space of time, an individual's fitness is more likely to be affected, and an increase in mortality may result. The fact that probability of transition from high mite load to low mite load is 0 in both sexes, indicates either that all mites drop off within a short space of time, or that during the mid-season when these high numbers were mostly observed, gaps between captures were insufficient to record the decline in numbers.

4.4.4. 2006 analysis

In 2006, survival was found to be reduced in the first interval following marking compared to all subsequent intervals. This suggests that marking has a negative effect or causes an increased tendency to emigrate following marking. This effect was suggested in females in 2005 but not in males. As the habitat was of lower quality and size than Latchmoor (see Chapter 3), it is possible that more emigration took place although no dispersal events were recorded. No effect of sex on survival rates was found, nor an interaction with the two-age-class effect as observed in the mites analysis. Recaptures rates did not differ between sexes in 2006 and were similar to

those for males in 2005. The site and population studied in 2006 were considerably smaller than those in 2005 (see Chapter 3). As the same level of sampling intensity was applied (albeit over a shorter number of hours per day), this allowed more time per individual resulting in more captures of females. Time to next recapture did not differ between males and females (Mann-Whitney U-test: U = 2035.5, P = .0155). Recapture probability again varied through time, probably due to stochastic environmental factors. Certainly the two noticeable dips in recapture probability at occasions 10 and 15 directly followed days of relatively heavy rain and strong wind, although these variables were not selected as improving model fit.

4.4.5. The effect of leg removal on survival

Removal of a leg was found to have no significant effect on survival in the subset of data taken from the 2006 population. The daily rate of survival from the best model (0.836), is similar to that estimated from the whole 2006 data set for intervals after the first (0.87), and the recapture rate (0.414) is in the lower range of those estimated across the study period (Fig. 4.8). MRR studies with concurrent genetic analyses often remove a leg for DNA extraction (e.g. Watts *et al.*, 2007a) assuming that survival and behaviour will remain unaffected. This result shows that the effect of leg removal is no greater than the effect of handling for marking, allowing greater confidence in this procedure for future studies. Damselflies in the field are frequently observed with one or more legs missing, and during the course of this study were observed copulating and feeding. Legs are used for perching, grooming and manipulating food, and as an individual has 6 legs, the loss of one or more is unlikely to hinder normal behaviours. The minimum number of legs recorded in the field was three; in all but one of these cases at least one leg on each side of the body remained and the individual's behaviour seemed unaffected.

4.4.6. Population size

Few studies have estimated population size in damselfly species, and none has used the Horvitz-Thompson method to incorporate recapture probabilities from a CJS-derived model. The calculated two-daily population size estimates are likely to be more reliable than the overall population size estimates (see below) and also allow comparison to other studies, none of which calculated total population size. More males than females were captured in 2005 (observed ratio of males to females = 1.24), and this could be either because fewer females were present in the population due to increased mortality or dispersal, or were harder to catch due to differences in behaviour and habitat choice. However, population size estimates for males and females were similar throughout the season in 2005 (Fig. 4.9), and the average two-daily sex ratio from these estimates is in fact female biased (0.78 : 1 from maturity analysis, 0.88 : 1 from mites analysis) as is the overall sex ratio (Table 4.9), suggesting that the main cause of the bias in observed sex ratio was due to different recapture rates (Figs. 4.3 & 4.7). Little difference was found in survival rates between mature males and females after the first capture interval in 2005 (Figs. 4.1 & 4.5). Males suffered greater mortality during the pre-reproductive period, but this should not have affected the sex ratio greatly due to the brevity of this period in males. It is interesting to note that the MNA method estimates more females than males towards the end of the season, when reduced damselfly numbers and increased proficiency of field workers ensures greater capture efficiency. This suggests more females may have been present throughout but not detected. However, this may also be due to increased survival or later emergence of females.

In 2006, no difference in survival between males and females was found and recapture rates were also almost equal (Fig. 4.8). However, the observed sex ratio was again male biased (1.8:1) and Horvitz-Thompson estimates indicated an average daily ratio of two males to one female (Table 4.9). This may be due to the difference in habitat between the two study sites. Latchmoor Brook is open and almost universally accessible, whereas Great Wheal Seton has a considerable amount habitat surrounding the main site which is inaccessible due to high walls, steep banks and thick gorse hedges.

These areas may be used by females during maturation, and as such any female mortality occurring in this dense vegetation, or females which emerged late in the study period and had not returned to the water to mate by the end, would have been effectively excluded from the study. Population rates can be biased if the study area is of a limited size (Barrowclough, 1978; Steen & Haydon, 2000) and while the Latchmoor Brook site allowed searching to occur over a larger range than *I. pumilio* occurred, in 2006 this was not possible and may have biased population size estimates towards males.

Table 4.9. Sex ratio of Latchmoor (2005) and Cornish (2006) *I. pumilio* populations. Observed sex ratio is calculated simply from the number of males and females captured and marked. The HT mean is the mean sex ratio across two-day capture occasions as calculated using the Horvitz Thompson method. HT total is the total population sex ratio calculated as described in the text. All values represent the ratio of males to one female.

Site	Observed	HT Mean	HT Total
2005	1.2	0.88	0.54
2006	1.8	2.0	1.5

Observed sex ratios in studies of damselfly populations are often male biased (e.g. Córdoba-Aguilar, 1994; Stettmer, 1996; Stoks, 2001a; 2001b), but the suggested reason for this varies between lower female recapture and survival rates. Anholt *et al.* (2001) found that *Coenagrion puella* males had greater recapture rates and that male *Ischnura elegans* had greater survival, both resulting in a male biased sex ratio. Anholt (1997) estimated male *Lestes disjunctus* to be 2.5 times as abundant as females when calculated using MRR methods, and nearly 8 times more abundant based on a transect walk count. This highlights the effect of recapture rate on transect walk estimates, and the resulting underestimation of female numbers. A detailed discussion of male biased sex ratios in damselfly populations is presented in Chapter 5.

However, it is interesting to note that as the only study in this thesis which attempted to survey all areas used by females, a female biased sex ratio is reported at Latchmoor based on HT estimates. Immature *I. pumilio* females are bright orange in colour and as such are much more visible than the

females of many other damselflies. This may result in more captures and sightings of females during this stage. In addition, many MRR studies have focused on the breeding sites within a habitat (e.g. Bennett & Mill, 1995; Stettmer, 1996; Conrad *et al.*, 1999; Stoks, 2001b; Rouquette & Thompson, 2005), whereas this study included the surrounding area. Males and females utilise these areas of taller vegetation at night for roosting (Hunger & Röske, 2001; Rouquette & Thompson, 2007b), but during the day they are utilised more by females (Foster & Soluk, 2006; Chapter 3). By including these areas, this study may provide a more accurate estimate of the sex ratio of the population, rather than that only at the breeding site. This suggests that the male biased sex ratios reported in most other studies may be at least partly due to underrepresentation of females in the data. However, when known recapture rates are less than 0.2 results from MRR models can be unreliable (O'Brien *et al.*, 2005). As the recapture rates for females estimated in these analyses were frequently below 0.2, it is possible that a degree of unreliability was present in the female parameters, and therefore population size estimates. Consequently, confidence in the calculated female biased sex ratio was reduced, as it may have resulted from modelling problems when capture rates were low.

The estimated total population size in 2005 (8723 ± 471) was larger than expected, at over four times numbers seen (2052), and this was due to a problem with the method of calculation. In the height of the flight season, it was not possible to mark all new individuals each day causing some to remain unmarked for a day or more. This was due to the unexpectedly large population size, and the limit imposed on number of captures by minimum handling time. Evidence for this reduced capture frequency is shown in both Fig. 4.3, illustrating reduced recapture rates during most of the central part of the study period; and in Fig. 4.13 (and accompanying ANOVA described above) which shows the difference in time to next capture for individuals captured in each of the first six weeks of the study. Consequently the proportion of unmarked individuals in the population would have increased during this time, whilst individual capture rates declined. This discrepancy is more likely to affect female estimates as the daily percentage of captured individuals which were unmarked was 20% higher in females on average. A

higher percentage of new females combined with lower recapture rates has caused the estimated female population size (5659 ± 440) to be almost twice that of males (3064 ± 166). Longer intervals between captures in females has been shown to positively bias female population size estimates when it constitutes temporary emigration from the water's edge (Kendall *et al.*, 1997; Stoks, 2001a).

The total population size in 2006 was estimated at 527 (±55), comprising 315 (±40) males and 212 (±38) females. The HT total method may perform better for the 2006 data as it is likely most new individuals were caught. It should be noted that as no estimates could be produced for occasions when no new individuals were caught, no contribution was made to the variance of the total population size estimate for that occasion. As such the Wald confidence intervals for total population sizes may be underestimated. Additionally where assumptions are violated (as is common in field studies) estimated variances are invalidated (Cook *et al.*, 1967) and are therefore provided for guidance only.

Estimation of population size using the HT method is both time consuming and subject to substantial sources of error. In almost all studies the strict assumptions of the MRR models used may not be entirely met. Confidence intervals for the HT estimates presented here are relatively large, and given the time required to produce them, a simpler method of estimation may be preferred. This study presents several series of daily population size estimates, produced using several methods, varying in their ease of data collection and calculation.

Transect walks cannot be expected to record all individuals in a population as the route represents only a linear section through the range of the population. However, in order to monitor changes in abundance, transect data must represent constant proportions of total population size (Harker & Shreeve, 2008). This study reports transect reliability (proportion of abundance estimated by HT) of between 0 and 53% which is remarkably similar to the range recorded for the butterfly *Lassiomata megera* (0 to 51%; Harker & Shreeve, 2008) but greater than that for the solitary bee *Andrena*

hattorfiana (5.5 to 23.4%; Larsson & Franzén, 2008). No negative relationship between the reliability of transect walks and HT estimates was found, indicating that transect walks were equally as reliable as an index regardless of density. Transect counts were correlated with HT estimates in this study ($r^2 = 0.36$, P = 0.008), although to lesser degree than the butterfly *Theclinesthes albocinta* measured across three sites and two years ($r^2 = 0.88$, P = 0.017; Collier *et al.*, 2008). In order to establish a predictive relationship between transect counts and population size data from multiple sites and years would be required (Pollard, 1977), and the strong correlation across sites and years reported by Collier *et al.* (2008) suggests that this may be highly informative. Two peaks in abundance were identified equally well by the two methods giving additional verification to both methods. The coefficient of variation for the Pollard walk counts of *I. pumilio* in this study was 69.5%. This value is relatively low within the range of values obtained for 22 species of butterflies (14.0% to 377.3%; Harker & Shreeve, 2008). However, the transect walks at Great Wheal Seton did not cover the entire flight season unlike the butterfly data and are therefore subject to less seasonal variation and are not directly comparable.

Diurnal variation in transect reliability was minimised in this study by restricting the timing of walks to between 11am and 3pm, which is the hottest time of the day when activity is greatest (Corbet, 1999; Ward & Mill, 2006). Annual indices can only be robust to variation in individual counts if effects such as temperature and time of day are minimised (Pollard, 1977; Harker & Shreeve, 2008). Furthermore, weekly sampling should be conducted on similarly warm days, or a series of observations made and one per week used as the official index based on similar weather conditions. Females were underrepresented in the transect walk data, which was due to the inaccessibility of many of the areas utilised by females. However, in more accessible habitat, with a well planned route incorporating water and surrounding vegetation, this should be resolved to a large degree.

Jolly-Seber estimates of population size proved a highly effective method of calculation when compared to HT estimates. Where MRR data is available and population size estimates are required without information on effects on

survival and recapture rates, the JS method as implemented in Simply Tagging (Pisces Conservation Ltd, 2003) or similar software may be preferred due their speed of use and low computational requirements. These estimates also have very small confidence limits in comparison to HT estimates although as the assumptions of the JS model are not fully met they are likely to be inaccurate (Cook *et al.*, 1967). Minimum number alive (MNA) values were also correlated with HT estimates. However, as the JS estimates were more highly correlated and less time consuming to compute, the JS method may be preferred.

4.4.7. Summary

In 2005, females exhibited greater survival rates, except in the first interval following capture, although no effect of sex was detected in 2006. The differences between survival rates in mature individuals after the first capture interval were small (mean difference: maturity analysis 0.015; mites analysis 0.039), and may not be considered to be biologically meaningful, as they equate to differences in longevity of less than half a day (0.24 and 0.31 days respectively). Survival rates decreased with increasing mite load and also declined both linearly with individual age and more generally through the flight season as the population aged. No effect of leg removal on survival was detected. Recapture rates were reduced in females in all analyses, although to a lesser degree in the Cornish population where capture effort per unit area was higher. Survival and recapture rates were also subject to stochastic variation through time due to weather.

The results of this study have implications for future study design when population sizes estimates and modelling of survival and recaptures rates are required. Study sites must be designated to cover all areas females (and males) may use for feeding, mating, ovipositing and all other behaviours, in order to ensure that females of all stages of maturity are well represented in the data. Alternatively a study must be designed whereby all areas of water can be watched in order to ensure every female is recorded when visiting to mate (Banks & Thompson, 1985b; Stoks, 2001a). Reduced female survival

in the first interval between captures suggests a potential handling effect and as such great care must be taken whilst marking.

This study demonstrates that transect walks represent a valuable method of monitoring population size in *I. pumilio* and more work should be done to establish a relationship across sites and years. This may be extended to other damselfly species and may be used as a tool to assist their conservation management. Efforts should be made to minimise bias due to weather conditions and site effects such as habitat size, but if well managed this method could be used to establish a successful monitoring program for damselflies such as that used by the Butterfly Monitoring Scheme. The method would be particularly suitable for large scale studies comparing many populations as it has much smaller effort-per-site requirements than MRR methods. O'Brien *et al.* (2005) recommend a concurrent, independent method of estimating demographic rates for comparison to MRR estimates, as a method of detecting biases therein. The results of this study indicate that transect walks may offer a reliable and relatively uncomplicated solution to monitoring changes in population size, provided efforts are made to reduce bias and to include areas utilised by both males and females.

Ischnura pumilio is declining in the UK and as such efforts should be made to manage the habitat at sites where the species persists according to sound management guidelines (Thompson *et al.*, 2003; Rouquette & Thompson, 2005; Chapter 3). The size of the Cornish population is considerably smaller than any of the *C. mercuriale* populations considered in Chapter 5 which is likely to have caused inbreeding and low levels of genetic variation which will be determined by forthcoming genetic analyses. Furthermore, along with many previous *I. pumilio* populations (Chapter 3), two of the surveyed Cornish sites have disappeared in the last ten years and at a third site (Rosewarne Mill) only three individuals were recorded in 2006. Reintroduction should be given careful consideration as isolated populations can be locally adapted (Watts *et al.*, 2004), which is particularly likely in these sites due to their industrial history and unusual habitat characteristics (Chapter 3). These sites would require substantial management if a

reintroduction scheme were to be successful, however this should be given serious consideration if the species is to persist in the Red River valley.

Chapter 5: Estimating survival and population size in *Coenagrion mercuriale* populations

5.1. Introduction

Coenagrion mercuriale is a conservation priority among European odonates, and is protected by law at a national and continental level (Wildlife and Countryside Act 1981; Bern Convention; European Community Habitats Directive). It is listed as rare in the British Red Data Book of Insects (Shirt, 1987), and until 2008 was the only odonate given priority status in the UK Biodiversity Action Plan (HMSO, 1994; 1995). It has a severely restricted range in the UK, occurring at only a few southern and western sites (Thompson & Watts, 2006; Watts *et al.*, 2007b). The species requires one of two increasingly fragmented habitat types in the UK: shallow, permanently-flowing, small streams and channels found in lowland, heathland streams over base-rich substrates, or calcareous streams in water meadow systems (Rouquette & Thompson, 2005). The species is also found in two fen sites in Oxfordshire and Anglesey.

C. mercuriale has been more comprehensively studied in the UK than in other European countries (Rouquette & Thompson, 2007a). Estimates suggest that the UK population has declined by 30% since 1960, principally due to anthropogenic factors such as changing land use (Watts *et al.*, 2004). The species has been well studied in England and Wales (e.g. Strange, 1999; e.g. Purse & Thompson, 2003c; Watts *et al.*, 2004; Purse & Thompson, 2005a; Watts *et al.*, 2007a; Rouquette & Thompson, 2007b) and several mark recapture studies have been undertaken (Purse *et al.*, 2003; Thompson & Watts, 2006; Rouquette & Thompson, 2007a). However, no study has made use of the MRR software available to produce estimates of survival and recapture probabilities, or in fact considered survival in this species at all.

This chapter reports analyses using recently developed modelling procedures to estimate survival and recapture probabilities in two populations of *C. mercuriale* in the south of England. The effects of sex, time and age were considered. The aim of this study was to provide information on the factors influencing survival rates in *C. mercuriale* populations in order to inform their conservation management. Further insights may be gained by comparing the factors influencing survival in *C. mercuriale* to those discussed in the previous chapter for *Ischnura pumilio*. The two species belong to the same family (Coenagrionidae), have similar habitat requirements (see Chapter 3; Thompson *et al.*, 2003; see Chapter 3; Rouquette & Thompson, 2005) and often inhabit overlapping areas within a site as adults (pers. obs.).

Estimates of population size in odonates are generally rare although attempts have been made in *C. mercuriale* (Thompson & Watts, 2006; Watts *et al.*, 2006). However none have made use of the HT method discussed in the previous Chapter, but have opted for less computationally intensive Jolly-Seber estimates based on male counts doubled to account for females. Given the uncertainty of a sex ratio equal to unity in damselfly species, this method may overestimate population size. Reliable population size estimates are important for rare species such as *C. mercuriale*, as along with estimates of genetic variation, they provide information on the relative ability of populations to adapt to environmental change and ultimately avoid extinction.

5.2. Methods

5.2.1. Study sites and populations

The data analysed were taken from two previous MRR studies of *C. mercuriale.* The first study area was located between Winchester and Southampton, Hampshire, England (SU464199). Here the species is found mainly on old water meadow ditches along the flood plain of the River Itchen. There were three main areas covered by this study between which no movement took place (Rouquette & Thompson, 2007a). These sites are, from north to south: Mariner's Meadow, Highbridge and the Lower Itchen Complex (LIC, comprising 5 sub-sites in Rouquette & Thompson, 2007a). Approximately 3km separates each adjacent site, comprising mostly unsuitable habitat including urban areas and intensive agriculture. A more detailed description of the Itchen sites is available in Rouquette & Thompson (2005). The study was conducted over 42 consecutive days commencing on 12th June 2001.

The second study took place at Beaulieu Heath, within the New Forest National Park, Hampshire, England (SZ336977). *C. mercuriale* is here found on a network of small flushes and runnels, which was divided in the original study into seven central areas and four peripheral sites. This study will consider these as two central areas and two peripheral sites. The central areas are Crockford and Peaked Hill, and the peripheral sites are Roundhill and Hatchet Stream. No movement occurred between any of these four sites, except one individual which moved from Crockford to Peaked Hill and was omitted from this analysis (Thompson & Watts, 2006). Sampling for this study took place every day for 5 weeks commencing the 11th June 2002.

Data collection followed similar methods to those described in Chapter 2. Searches were performed along linear sections of stream in the Itchen study area, and wider searching techniques were employed at Beaulieu. This is partly due to the nature of the habitat in the two areas: the study area at Itchen comprised linear stretches of stream and ditch, only some of which were accessible, whereas the Beaulieu site covered a complex network of

wet and dry areas. In the Itchen study, females were only captured when in copula, but lone females were also recorded at Beaulieu. Numbers of individuals captured, marked and released are summarised in Tables 5.3 and 5.4 for Itchen and Beaulieu respectively. Some individuals were omitted due to missing data (27 individuals from Itchen and 16 from Beaulieu). Capture histories for each individual were constructed and modelling of survival and recapture probabilities was conducted using the program MSURGE (Choquet *et al.*, 2004). Capture histories from both locations were grouped into two day intervals due to the data being too sparse in single day interval format.

5.2.2. Models, assumptions and model selection

Procedures for goodness of fit (GOF) testing and model selection are equivalent to those outlined in Chapter 4. As no transition between sites occurred, single state models were used to analyse the Beaulieu and Itchen data sets. Modelling of both data sets therefore proceeded from the CJS model (described with assumptions in Chapter 4), modified as indicated by failure of components of the GOF test for the CJS model in U-CARE. The variance inflation factor, $\hat{c}$, was calculated as the ratio χ^2/df using pooled chi squared statistics and degrees of freedom from GOF tests (Lebreton *et al.*, 1992).

5.2.3. Model specification

It was expected that survival and recapture probabilities could be dependent on sex (*s*) and/or time (*t*) and/or site (location - *l*). An *a priori* set of models was defined to include and exclude each of the considered effects in an interactive manner. Additive effects were also included where they seemed biologically plausible. A degree of *post hoc* model simplification was then undertaken, to ensure the selected effects should be included in the best model. A list of all models considered for each analysis appears in Appendix 2.

5.2.4. Population size estimates

Estimates of recapture probabilities from the best models in each analysis were used to calculate estimates of population size using a Horvitz-Thompson type estimator (Horvitz & Thompson, 1952; McDonald & Amstrup, 2001), following the methods described in the previous chapter.

5.3. Results

5.3.1. Itchen

Initial GOF tests revealed that the CJS model $\varphi_t p_t$ was strongly rejected for the Itchen data (χ^2 = 540.526, P < 0.0001, df = 242). Examination of the component tests revealed that males at Mariner's Meadow and LIC failed test 3.SR (Mariner's Meadow: χ^2 = 61.171, P < 0.0001, df = 13; LIC: χ^2 = 45.878, P = 0.0003, df = 18), indicating a transience or marking effect in these groups. Males at Mariner's Meadow and LIC also failed test 2.CT, which tests for trap dependence (Mariner's Meadow: χ^2 = 45.107, P < 0.0001, df = 10; LIC: χ^2 = 105.406, P < 0.0001, df = 16). A *z* test performed in U-care indicated trap-happiness rather than trap-shyness in both cases. These initial structural failures were accounted for by including a two-age-class effect on survival (Brownie & Robson, 1983) to account for a transience or marking effect, and a trap dependence effect allowing recapture probabilities to vary between individuals captured at the previous capture occasion and those not (Pradel, 1993; Gimenez *et al.*, 2003). Structural modifications were applied only to the groups that failed the relevant component test, i.e. males at Mariner's Meadow and LIC in both cases.

Once the indicated transience and trap dependent effects had been accounted for, the GOF tests remained highly significant (χ^2 = 282.964, P < 0.0001, df = 185) suggesting additional lack of fit. The two remaining subcomponents of the GOF test (3.Sm and 2.CL) were also failed by males at Mariner's Meadow and LIC. These subcomponents test for a difference in the expected time of reencounter between new and old individuals, and for a difference in the expected time of reencounter between those individuals encountered and not encountered at a given occasion respectively. These structural deviations do not have standard alternative models which account for their lack of fit. Therefore, they must be at least partially accounted for by modifying the variance inflation factor, ĉ, in MSURGE to the calculated value of 1.53 (Pradel *et al.*, 2005). Model selection should proceed from a

model which adequately fits the data, but in cases like this that is not possible. However the most important (R. Pradel, pers. com.) structural deviations from the CJS model were accounted for (transience and trap-dependence) and the ĉ value adjusted, allowing model selection to be undertaken, although the results must be interpreted with caution. Lebreton *et al.* (1992) state that when it is not possible to determine the subtle structure of the data which causes lack of fit, it is appropriate to incorporate this residual structure into the model error component. Modifying the variance inflation factor, ĉ, fulfils this by inflating the standard errors of the estimates to reflect the level of uncertainty about the true parameter values. The formula for calculating AIC is also modified to give a "quasi-likelihood adjust AIC" or $QAIC_c$. The formula for $QAIC_c$ is:

$$QAIC_c = \frac{-2\ln(L)}{\hat{c}} + 2K + \frac{2K(K+1)}{M-K-1}$$

This modification favours models with fewer parameters as the value of ĉ increases (Cooch & White, 2006).

Table 5.1. The best 10 models based on AIC values for the Itchen data. Number of parameters (NP), model deviance, Akaike information criterion (AIC), AIC differences (ΔAIC) and AIC weights. Subscripts – *l* = location/site, *s* - sex, *t* - time. Two-age-class and trap-dependence effects were included in the general model but were not featured in the final model.

Model	DF	Dev	AIC	ΔAIC	AIC weight
$\varphi_{t+l}\, p_{t*l+s}$	79	20709.66	13693.73	0.000	0.6277
$\varphi_{t+s+l}\, p_{t*l+s}$	80	20709.63	13695.7	1.979	0.2334
$\varphi_{t}\, p_{t*l+s}$	77	20721.5	13697.47	3.742	0.0967
$\varphi_{t+s*l}\, p_{t*l+s}$	82	20708.73	13699.12	5.393	0.0423
$\varphi_{t*s+l}\, p_{t*l+s}$	98	20688.73	13718.05	24.320	0.0000
$\varphi_{t+l}\, p_{t*s*l}$	124	20640.86	13738.76	45.033	0.0000
$\varphi_{t+s+l}\, p_{t*s*l}$	125	20639.84	13740.09	46.367	0.0000
$\varphi_{t}\, p_{t*s*l}$	122	20652.42	13742.31	48.587	0.0000
$\varphi_{t+s*l}\, p_{t*s*l}$	127	20637.86	13742.79	49.069	0.0000
$\varphi_{t+s*l}\, p_{t*l}$	127	20637.86	13742.8	49.069	0.0000

Values of deviance, AIC, difference in AIC, AIC weight and number of parameters for the best 10 models tested are presented in Table 5.1. In total, 64 models were tested and are listed in Appendix 2. The model selected by AIC as best explaining the data was $\varphi_{t+l}\, p_{t*l+s}$. Model subscripts in this

analysis were defined as: *a2* – two-age-class effect, *l* - location/site, m - trap-dependence, *s* - sex, *t* - time. The AIC weights show that this model had over twice as much support in the data as the next model $\varphi_{t+s+l}\, p_{t*l+s}$.

The final model indicates that survival rates vary through time and that site has an additive effect (Fig. 5.1). Error bars are omitted from some figures in this chapter to aid interpretation, but the level of error in the estimates as calculated by MSURGE was generally large. Highbridge, had slightly higher survival rates than Mariners Meadow (mean difference = 0.049) and LIC (mean difference = 0.043), where rates were almost identical (mean difference = 0.005). The calculated two-daily survival rates from this study may be converted to estimated daily survival rates by taking the square root (Cook *et al.*, 1967), assuming that survival is approximately constant across two-day intervals. The average survival rate for *C. mercuriale* at Itchen was 0.62, which corresponds to a daily rate of 0.78. This value was converted into an average life expectancy of 4.02 days using the method recommended in Cook *et al.* (1967). Recapture rates also varied through time and between sites but in this case an interaction was indicated, and a further, additive effect of sex whereby males were almost three times as likely to be captured as females (Fig. 5.2).

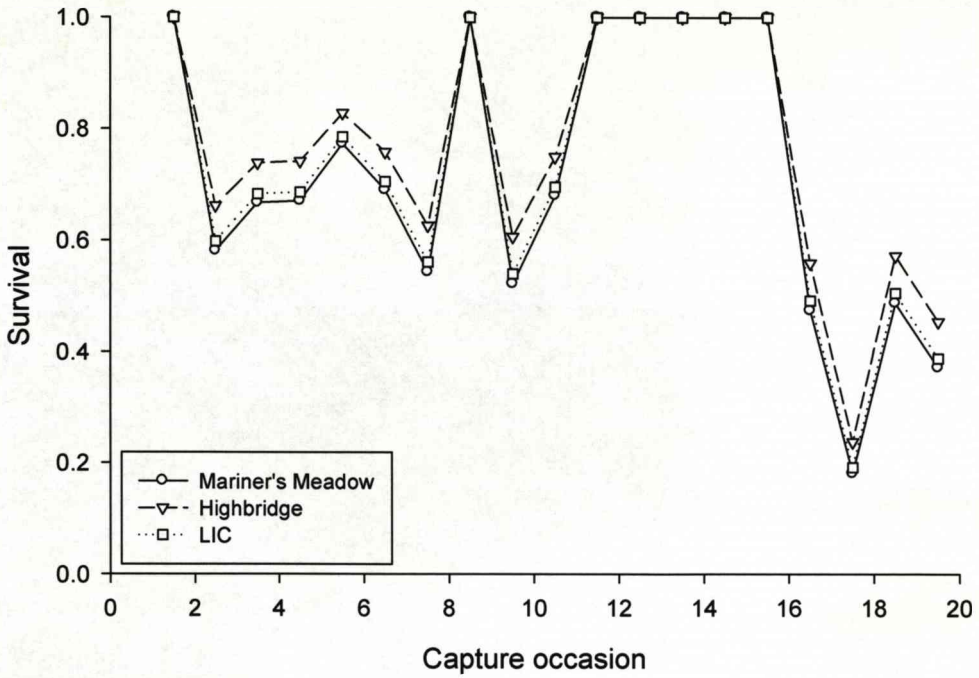


Figure 5.1. Maximum likelihood estimates of survival probability for C. mercuriale at Itchen. Lines connecting values for the same site are intended for orientation only. Estimates were calculated by MSURGE using the model $\varphi_{t+l}\, p_{t*l+s}$.

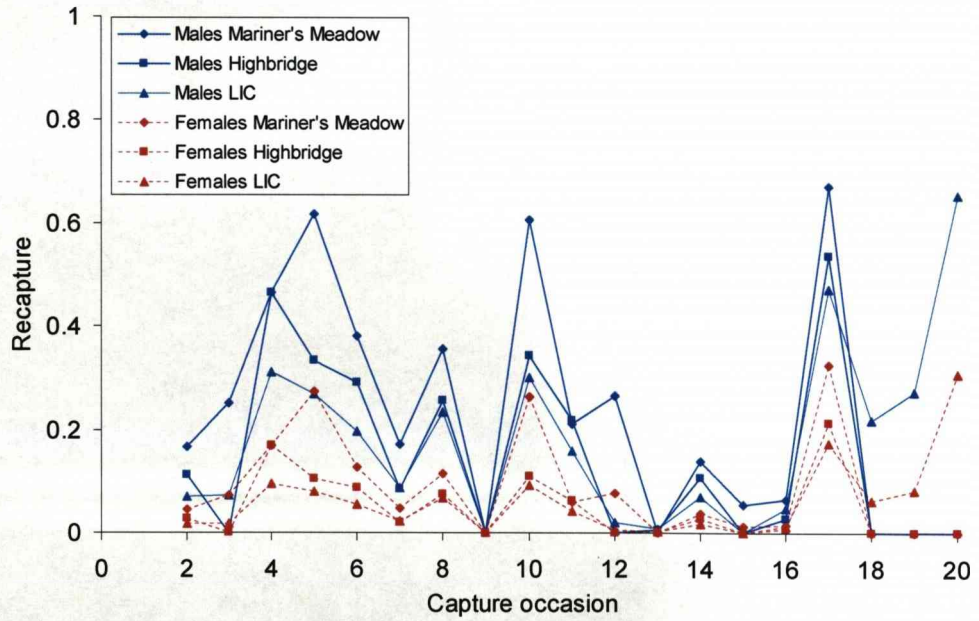


Figure 5.2. Maximum likelihood estimates of recapture probability for *C. mercuriale* at Itchen calculated by MSURGE using the model $\varphi_{t+l}\, p_{t*l+s}$. Lines connecting values for the same site are intended for orientation only.

5.3.2. Beaulieu

Initial GOF tests rejected the CJS model $\varphi_t\ p_t$ for the Beaulieu data (χ^2 = 547.48, P = 0.0001, df = 432). Examination of the component tests revealed that males at Roundhill failed test 3.SR (χ^2 = 29.11, P = 0.0156, df = 15), indicating a transience or marking effect in this group. In addition, males at Crockford and Peaked Hill failed test 2.CT, which tests for trap dependence (Crockford: χ^2 = 23.63, P = 0.05079, df = 14; Peaked Hill: χ^2 = 69.46, P < 0.0001, df = 14). U-care again indicated trap-happiness rather than trap-shyness in both cases. To account for structural failure, a two-age-class effect on survival and a trap dependence effect on recapture were again included for the groups that failed the relevant component tests. Once the indicated transience and trap dependent effects had been accounted for, the overall GOF test was non-significant (P = 0.0992) indicating that the modified umbrella model fitted the data adequately. The calculated value of ĉ was 1.09 indicating slight residual overdispersion, therefore the value was modified in MSURGE to account for this when calculating standard errors and $QAIC_c$ values.

Table 5.2. The best 10 models based on AIC values for the Beaulieu data. Number of parameters (NP), model deviance, Akaike information criterion (AIC), AIC differences (ΔAIC) and AIC weights. Subscripts – *l* = location/site, *s* - sex, *t* - time. Two-age-class and trap-dependence effects were included in the general model but were not featured in the final model.

Model	DF	Dev	AIC	ΔAIC	AIC weight
$\varphi_{t+s+l}\ p_{t*l+s}$	84	34153.531	31501.515	0.000	0.5557
$\varphi_{a2[1]*t+l}\ p_{t*l+s}$	85	34153.270	31503.275	1.760	0.2305
$\varphi_{t+l}\ p_{t*l+s}$	83	34157.821	31503.451	1.936	0.2111
$\varphi_{t+s+l}\ p_{t*[l+s]}$	99	34132.610	31512.321	10.806	0.0025
$\varphi_{a2[1]*t+s}\ p_{t*l+s}$	83	34172.914	31517.297	15.783	0.0002
$\varphi_{t*l+s}\ p_{t*l+s}$	125	34082.919	31518.733	17.218	0.0001
$\varphi_{a2[1]+t*l}\ p_{t*l+s}$	126	34084.174	31521.885	20.370	0.0000
$\varphi_{t}\ p_{t*l+s}$	80	34203.610	31539.459	37.944	0.0000
$\varphi_{a2[1]*t}\ p_{t+s+l}$	38	34323.430	31565.385	63.870	0.0000
$\varphi_{a2[1]*t}\ p_{t*s*l}$	137	34116.417	31573.466	71.951	0.0000

Values of deviance, AIC, difference in AIC, AIC weight and number of parameters for the best 10 models tested are presented in Table 5.2. In total, 81 models were tested and are listed in Appendix 2. The model selected by AIC as best explaining the data was $\varphi_{t+s+l}\ p_{t*l+s}$. Model subscripts in this

analysis were defined as: *a2* – two-age-class effect, *l* - location/site, m - trap-dependence, *s* - sex, *t* - time. The AIC weights show that the best model was over twice as well supported as the next model $\varphi_{a2[15]*l}\, p_{t*l}$.

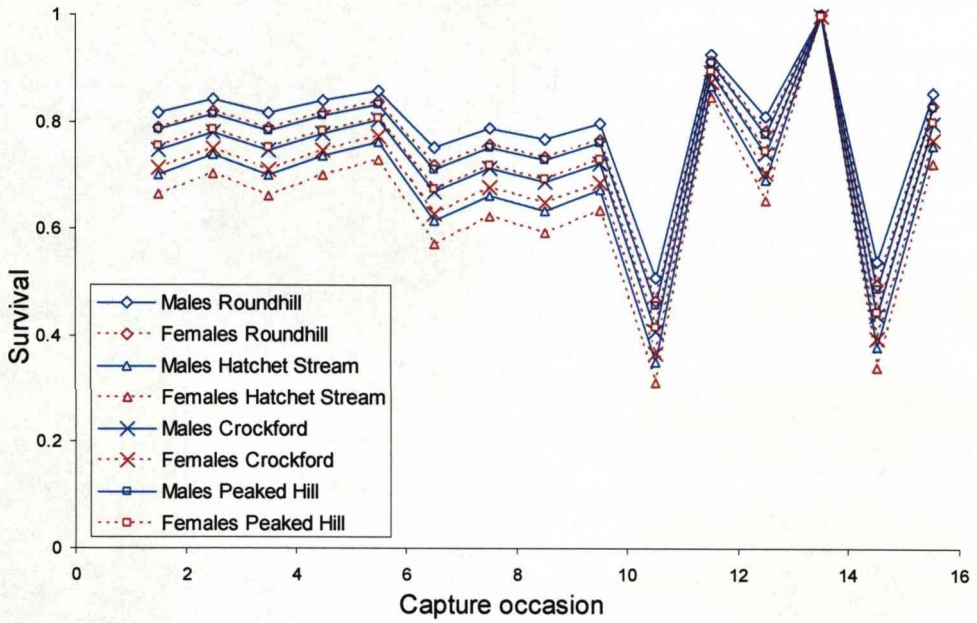


Figure 5.3. Maximum likelihood estimates of survival probability for *C. mercuriale* at Beaulieu calculated by MSURGE using the model $\varphi_{t+s+l}\, p_{t*l+s}$. Lines connecting values for the same sex and site are intended for orientation only. Survival is greatest at Roundhill, followed by Peaked Hill, then Crockford and Hatchet Stream has the lowest rate. Female survival is lower than that of males at each site.

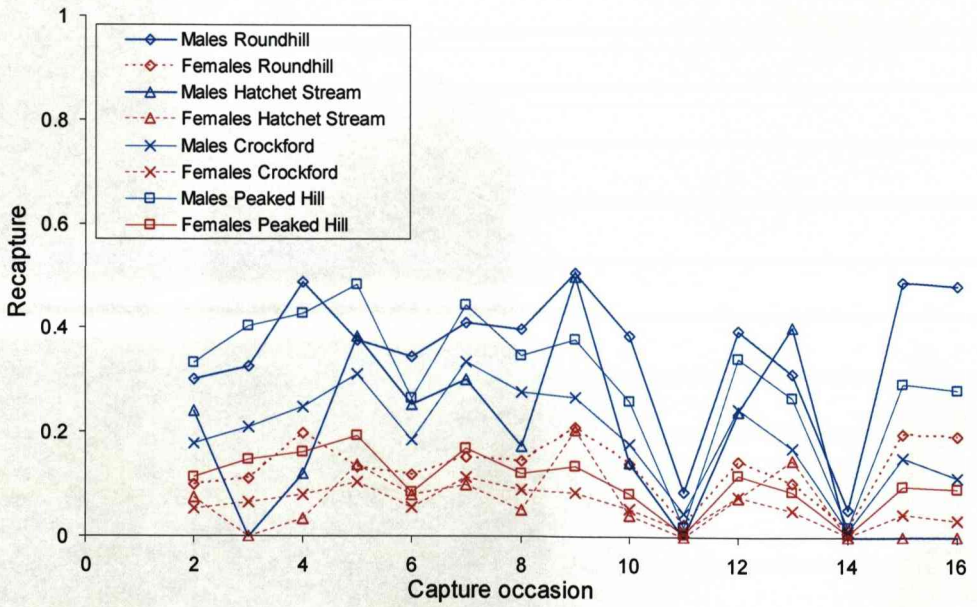


Figure 5.4. Maximum likelihood estimates of recapture probability for *C. mercuriale* at Beaulieu calculated by MSURGE using the model $\varphi_{t+s+l}\, p_{t*l+s}$. Lines connecting values for the same sex and site are intended for orientation only.

This indicates that again survival rates vary through time and that site and sex each have additive effects (Fig. 5.3). Crockford and Peaked Hill had almost identical, intermediate survival rates; Hatchet Stream the lowest and Roundhill the largest (Fig. 5.4). Females had a slightly lower survival rate than males. The average survival rate for male *C. mercuriale* at Beaulieu was 0.72 and for females 0.69, which correspond to daily rates of 0.85 (6.15 days) and 0.83 (5.37 days) respectively. These survival values are very similar and the difference may be considered negligible, although the inclusion of the effect of sex was significant when tested using LRT (χ^2 = 4.29, P = 0.0383, df = 1). Recapture rates were again subject to interactive effects of time and site with an additive effect of sex (Fig. 5.4).

5.3.3. Population size estimates

Population size estimates calculated for the combined Itchen sites are shown in Figure 5.5. Numbers are shown separately for males and females so that predicted sex ratio may be discussed. The maximum estimated number occurred at capture occasion 16 which represents the 12th and 13th July 2001. An estimated 12495 (± 967) individuals were present in the population during this period, comprising 9487 (± 875) males and 3009 (± 1002) females. The overall population size for the season calculated using the Horvitz-Thompson estimator with new individuals (see Chapter 4) was 71976 (± 5029), comprising 52142 (± 4518) males and 19834 (± 2209) females (all estimates presented with 95% Wald confidence intervals). Observed and estimated overall population sizes for the *C. mercuriale* population at Itchen are summarised in Table 5.3. Where no individuals were recorded on a capture occasion, an HT estimate or variance cannot be calculated and therefore no data point is shown on the relevant chart. In addition, where parameters were estimated close to the boundary with standard errors so large that confidence intervals included negative values; the estimate for that date was also omitted.

Table 5.3. Total numbers of *C. mercuriale* in the Itchen population. Observed (Obs) values (total numbers marked and released) and Horvitz-Thompson (HT) estimates are presented with their 95% Wald confidence intervals.

Site	Mariner's Meadow		Highbridge		LIC		Total	
	Obs	HT	Obs	HT	Obs	HT	Obs	HT
Males	1613	3046 ±244	1101	2874 ±277	7933	46222 ±4503	10647	52142 ±4518
Females	236	1688 ±376	72	706 ±212	864	17440 ±2167	1172	19834 ±2210
Total	1849	4734 ±448	1173	3580 ±349	8797	63662 ±4997	11819	71976 ±5029

Figure 5.5 shows the population size estimates for the combined Beaulieu populations. Numbers are again shown separately for males and females as an effect of sex on recapture rates was retained in the best model for these data. The highest estimate of 7347 (± 337) individuals occurred on day 9 (4707 ± 196 males and 2640 ± 279 females). The overall estimated population size for the season was 44722 (± 1621), comprising 27175 (± 931) males and 17547 (±1328) females (all estimates presented with 95% Wald confidence intervals). Observed and estimated population sizes for the *C. mercuriale* population at Beaulieu are summarised in Table 5.4. As before, estimates from dates with no records or where the recapture parameter was estimated on the boundary with a large standard error were omitted.

Table 5.4. Total numbers of *C. mercuriale* in the Beaulieu population. Observed (Obs) values (total numbers marked and released) and Horvitz-Thompson (HT) estimates are presented with their 95% Wald confidence intervals.

Site	Roundhill		Hatchet Stream		Crockford		Peaked Hill		Total	
	Obs	HT	Obs	HT	Obs	HT	Obs	HT	Obs	HT
Males	2836	3600 ±211	247	617 ±110	3777	11539 ±772	7312	11419 ±463	14172	27175 ±931
Females	517	2490 ±242	71	645 ±199	531	6567 ±1200	1217	7845 ±476	2336	17547 ±1328
Total	3353	6090 ±321	318	1262 ±227	4308	18106 ±1427	8529	19264 ±664	16508	44722 ±1621

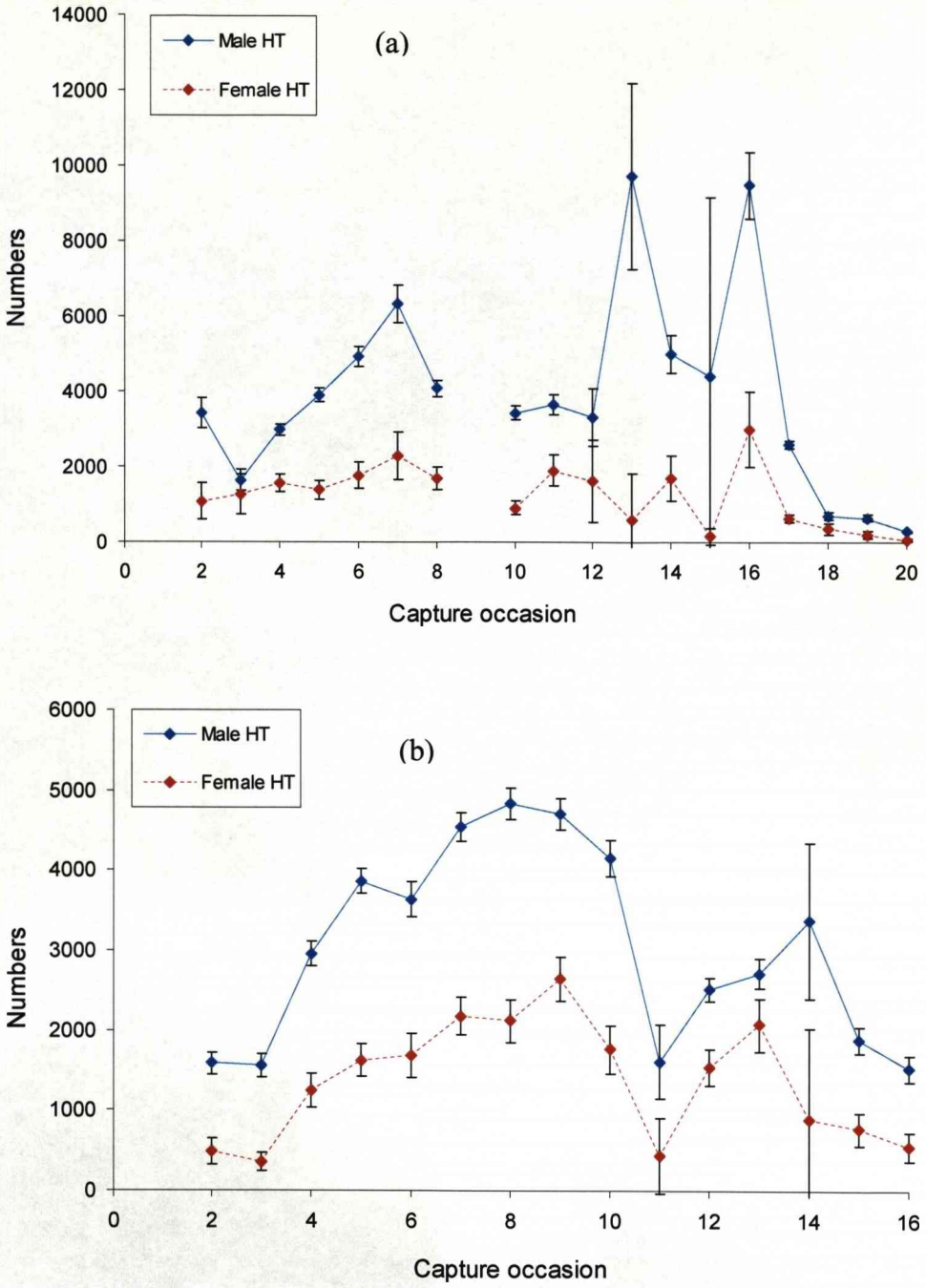


Figure 5.5. Number of adult male and female *C. mercuriale* in the Itchen (a) and Beaulieu (b) populations estimated using the Horvitz-Thompson (HT) method and parameter estimates from the models $\varphi_{t+1}\ p_{t*1+s}$ and $\varphi_{t+s+1}\ p_{t*1+s}$ respectively. Lines connecting values for the same sex are intended for orientation only. Errors bars represent 95% Wald confidence intervals.

5.5 Discussion

5.5.1. Assumptions

Initial departure from the assumptions of the CJS model due to a transience or marking effect was accounted for by fitting a two-age-class model. Further structural modification of the umbrella model was necessary to incorporate a trap dependence effect, allowing recapture probabilities to vary between individuals captured at the previous capture occasion and those not. Once these major structural features of the data had been accounted for in the umbrella model, there was evidence for additional lack of fit in both data sets, indicated by values of ĉ greater than 1. Furthermore, in the case of the Itchen data, GOF tests indicated significant lack of fit of the modified umbrella model which could be due to further structural deviations. Failure of GOF subcomponent tests (3.Sm and 2.CL) by males at Mariner's Meadow and LIC indicated structural deviations from the CJS model which have no standard alternatives.

Overdispersion or lack of fit may be caused by any combination of structural failure of the model, heterogeneity of survival or recapture rates and non-independence of individuals fates (Lebreton *et al.*, 1992). As the calculated values of ĉ were greater than 1, it is possible that these assumptions were violated to some degree, although the structural failure indicated by the failure of GOF subcomponents 3.Sm and 2.CL is likely to be the cause of most of the remaining lack of fit to the Itchen data. Some groups of females were omitted from the study at Itchen and this may have had implications for the final model which are discussed below. However, the calculated values of ĉ were relatively low (1.53 for Itchen and 1.09 for Beaulieu). Lebreton *et al.* (1992) and Cooch & White (2006) recommend that model selection should not be undertaken from starting models with values of ĉ greater than 3. Therefore the lack of fit is not severe and should be well accounted for by the modified $QAIC_c$ values.

The suggested trap dependence may be due the increased likeliness of males to be present at the water on subsequent days when mature, which may

result in higher recapture probabilities in males captured on the previous day. Females on the other hand visit only when ready to lay eggs (Banks & Thompson, 1987; Anholt, 1992; Stoks, 2001a). Fincke (1986) reported that female *Enallagma hageni* visited water on average every 5.2 days after maturing a clutch of eggs. However, although trap dependence was indicated by the initial GOF tests, it did not feature in the final models selected by AIC. This suggests that the effect size was too small to warrant its inclusion in the best models given the number of additional parameters it required. The indication of trap dependence in male *C. mercuriale,* but not *I. pumilio,* could be because mature *C. mercuriale* males spend the majority of the active part of the day at the water, whereas *I. pumilio* males spend at least some time mate searching and mating away from water (see chapter 3). However this has not been directly studied in *C. mercuriale*.

If only the areas of water surveyed are considered to be the MRR study site and not the surrounding vegetation, females returning to surrounding vegetation between bouts of oviposition violate the assumption that all emigration is permanent (Stoks, 2001a). Kendall *et al.* (1997) showed that differences in temporary emigration probability such as those between females that have recently oviposited and those that have not, result in negatively biased capture probabilities. Longevity of *Pyrrhosoma nymphula* was underestimated using data collected only at water, when compared to data collected from the hinterland also (Bennett & Mill, 1995). Temporary emigration may also be thought of as trap shyness, as a female captured on one occasion is less likely to be captured on the next (Stoks, 2001a). It is unclear why this effect was not detected by GOF tests, although the results showed that trap happiness was much less marked in females, and in two cases trap shyness was indicated although the effect was not significant (females at Mariner's Meadow and Peaked Hill). Too few females may have been present in the Itchen data to detect an effect, due to their capture only when in copula. Some GOF test components returned no result for females at Highbridge, Itchen and Hatchet Stream, Beaulieu. This is because the data for these groups was so sparse due to low numbers, that no test could be conducted.

5.5.2. Itchen

Estimated survival rates varied greatly with time in this analysis (Fig. 5.1). On several occasions the estimated two-day survival rate approached 1, and this coincides approximately with those occasions when capture rates and numbers seen were relatively low (Figs. 5.2 and 5.6). Due to periods of inclement weather, on one two-day capture occasion no individuals were recorded at any site, and on 8 further occasions no records were obtained at 1 or more sites. When no individuals are recorded on a given occasion, it becomes impossible to separately estimate the survival rates for the intervals either side of this occasion and lack of data can cause parameters to be estimated on a boundary (Viallefont *et al.*, 1998). The additive nature of the survival component of the selected model has forced all estimates at the affected capture occasions to be estimated close to 1, as opposed to only the rates for the affected sites. For these reasons the survival estimates close to 1 should be ignored as their values are unlikely to represent the true values for the population at that time. Disregarding those values close to 1, rates decreased somewhat during the season, appearing to be lower after the period of low numbers of observations (occasions 12 to 16). As the low capture rates were caused by bad weather, it seems likely that survival was affected during the following days. Lower temperatures prevent odonates from foraging due to decreased activity (Corbet, 1999; Ward & Mill, 2006), and bad weather such as wind and rain may physically damage damselflies, and has been found to decrease survival in *Ischnura denticollis* (Córdoba-Aguilar, 1993).

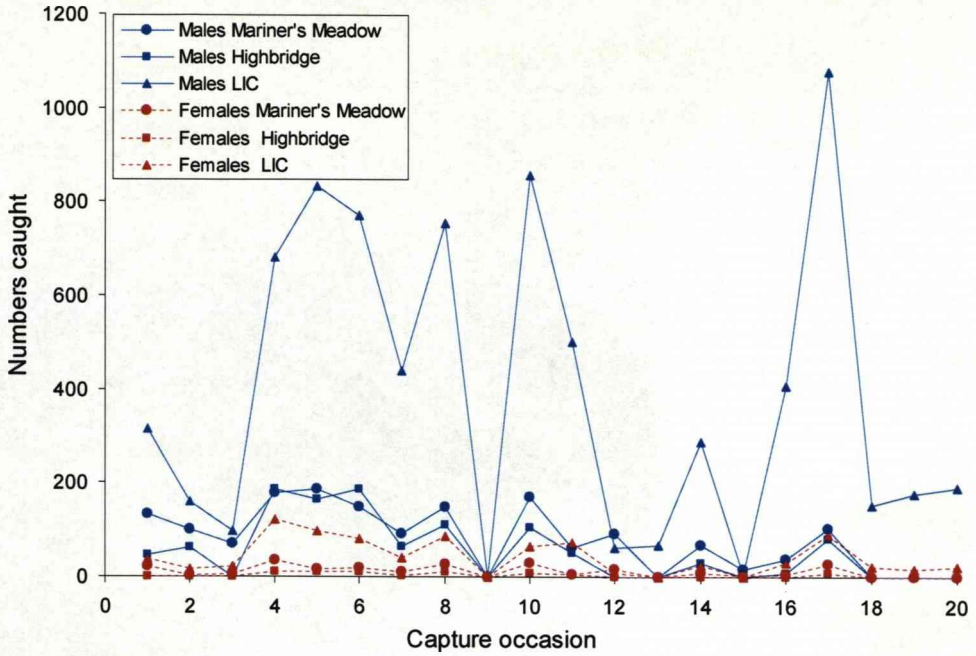


Figure 5.6. Number of adult male and female *C. mercuriale* captured or recaptured at each of the Itchen sites. Lines connecting values for the same sex are intended for orientation only.

The only other factor found to influence survival rates significantly in the Itchen population was site. The central site, Highbridge, had slightly higher survival rates than Mariner's Meadow to its north (mean difference = 0.049) and LIC to its south (mean difference = 0.043), where rates were almost identical (mean difference = 0.005). Habitat quality at Mariner's Meadow was good (Rouquette & Thompson, 2005) but it was the most genetically distinct of the sites and also had high levels of kinship among individuals, suggesting that it may have suffered the effects of genetic isolation and inbreeding (Watts *et al.*, 2004) which can negatively affect survival rates. Conversely, the larger LIC had more genetic variation but also more varied habitat, and survival may be reduced where habitat is poor. Highbridge had intermediate levels of both habitat quality and genetic variation, therefore a combination of environmental factors, management or genetics at that site may have resulted in slightly increased survival.

Recapture rates also varied considerably through the study period. Where periods of bad weather reduced numbers seen, recapture rates were inevitably reduced also. Insects require heat from the sun in order to fly, and on days of low temperature and dense cloud cover become notoriously

difficult to locate. Much, if not all, of the observed stochastic variation in recapture rates is likely to be due to environmental conditions, as sampling effort per unit area was constant throughout the study. Probability of recapture also varied between sites, with Mariner's Meadow generally having the highest rates and LIC the lowest. Mariner's Meadow is the most northerly of the three sites and also the most isolated. Rouquette & Thompson (2007a) found that distances moved by *C. mercuriale* at Mariner's Meadow were shorter than all other sites covered by the study, and also that this site contained sections with the highest densities. A high density of less mobile individuals would facilitate capture, and could therefore be the cause of the increased capture rates at Mariner's Meadow. LIC is a much larger area, and comprises 5 sub-sites that were considered separately in the original study, but grouped here due to recorded movement between them. Therefore, LIC contained much more varied habitats and densities than either Mariner's Meadow or Highbridge, which may have resulted in a lower capture rate at that site overall.

A significant effect of sex on recapture rates was also detected. Males were almost three times as likely to be captured as females, as might be expected given the known difficulty of locating female damselflies during the long periods they spend maturing and feeding away from water. Furthermore, in this study, reduced recapture rates in females were more likely, as females were only captured in copula, which cannot happen every day as females must mature new eggs after each bout of oviposition (Fincke, 1986; Banks & Thompson, 1987; Stoks, 2001a). An effect of sex on recaptures rates is common in MRR studies of damselflies (e.g. Parr & Parr, 1972; Waage, 1972; Van Noordwijk, 1978; Bennett & Mill, 1995; Anholt, 1997; Andres & Cordero Rivera, 2001) due to this differential habitat use between the sexes whereby females spend more time concealed in surrounding vegetation. This is amplified by the difference in colouration between males and females resulting in males being more conspicuous. *Coenagrion mercuriale* females are similarly cryptically coloured to *I. pumilio* females, and males are even more brightly coloured than *I. pumilio* males, and certainly more conspicuous than conspecific females.

During the data collection period for the Itchen study the pattern of rainy days resulted in a two-day capture occasion on which no individuals were recorded at any site, and there were further occasions on which no records were obtained at 1 or more sites. This causes some parameters in the model to be non-estimable, and can further bias parameter estimates and cause problems with model selection using AIC (Viallefont *et al.*, 1998). In addition, some parameters were estimated on the boundary (close to 0 or 1) which can be either due to lack of data or true values being close to 0 or 1. This causes irregularities in the likelihood function and makes model selection using AIC unreliable (Viallefont *et al.*, 1998). This and the violation of assumptions discussed above could have introduced bias into the estimates produced from this model, and as such they should be interpreted with caution.

5.5.3. Beaulieu

The variation through time in *C. mercuriale* survival rates at Beaulieu (Fig. 5.3) was much more pronounced later in the season (after occasion 10). Numbers caught were also more variable during this time due to two periods of bad weather, resulting in no individuals being recorded at Hatchet Stream on four of the two-day capture occasions (Fig. 5.7). As described previously, variation in temperature and other weather variables can lead to variation in survival and recapture probabilities (e.g. Córdoba-Aguilar, 1994; Ward & Mill, 2006) and the absence of any captures on an occasion leads to problems modelling survival rates on neighbouring occasions (Viallefont *et al.*, 1998). These factors are likely to be the major cause of the fluctuations in survival rates in the later part of the study. These fluctuations aside, survival rates also decrease generally as the season progresses. This was also found in the Itchen population and in *I. pumilio* (Chapter 4), and has also been reported in the butterfly *Parnassius clodius* (Auckland et al., 2004). This seasonal decline in survival is to be expected as each study period covers the main flight season of the relevant species (Smallshire & Swash, 2004), towards the end of which most individuals had emerged and survival rates would have decreased as the population aged.

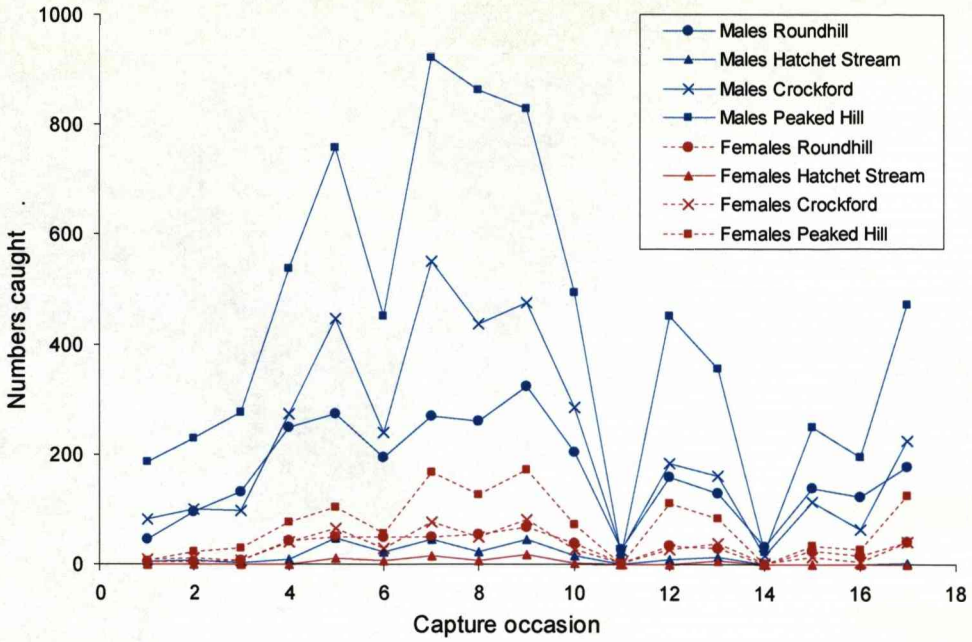


Figure 5.7. Number of adult male and female *C. mercuriale* captured at each of the Beaulieu sites. Lines connecting values for the same sex are intended for orientation only.

Some difference in survival probability between sites was also indicated: The two larger sites (Crockford and Peaked Hill) had almost identical, intermediate survival rates; the smallest site (Hatchet Stream) had the lowest survival probability and Roundhill the largest (Fig. 5.4). Thompson & Watts (2006) were unable to distinguish these sites genetically, although genetic differentiation does exist in *C. mercuriale* across the New Forest as a whole. This suggests that they were effectively one population or metapopulation and had equal levels of genetic variation, though no dispersal between them was detected. The smaller, apparently more isolated sites were not suffering the effects of inbreeding or genetic drift and differences in survival cannot, therefore, be attributed to genetic factors. Differences in management, habitat quality or levels of predation may be responsible for the observed differences (largest mean difference between sites = 0.21 between Roundhill and Hatchet Stream).

Females had a slightly lower survival rate than males at the same time and location in this analysis. This contradicts the results for *I. pumilio* presented in Chapter 4, which showed that females at Latchmoor had slightly greater survival rates than males except in the first interval after marking when they

suffered greater mortality. However, the Cornish *I. pumilio* and Itchen *C. mercuriale* populations showed no difference in survival rates. The average daily survival rate for male *C. mercuriale* at Beaulieu was 0.85 and for females 0.83. These values are very similar and the difference may be considered negligible (as was the case in mature *I. pumilio* at Latchmoor), leading to the conclusion that sex does not have a biologically meaningful effect on sex in mature *C. mercuriale.* This is supported by the absence of an effect of sex on survival rates in final model for the Itchen data.

However, the effect of sex on survival in *C. mercuriale* is small, and evidence from the literature suggests that the effect of sex on survival is not always present in damselfly populations, particularly in those studies which account for differences in recapture probabilities. Of the published studies which use similar MRR modelling techniques to those used here: Cordero Rivera & Andres (1999) found no difference in survivorship between the sexes in *I. pumilio*, as did one study reported in Chapter 4; Andres & Cordero (2001) found that survival rates in mature *Ceriagrion tenellum* were also similar, but that males suffered greater mortality during the pre-reproductive period than females in field and laboratory populations; however, Anholt *et al.* (2001) reported that daily survival was considerably different in *I. elegans* (males = 0.81 and females = 0.58).

Using other methods, Hafernik & Garrison (1986) found survival rates in *Ischnura gemina* to be equal in a population where they were able to mark almost all individuals, but found male survival (0.96) to be higher than female (0.86) in another study of the same species (Garrison & Hafernik, 1981). Similarly, Stoks (2001b) reported that mature male *Lestes sponsa* had higher survival rates than mature females in one population but not in another. Females may experience greater mortality due to the greater energy demands of maturing successive batches of eggs. This may necessitate increased foraging activity allowing more exposure to predation (Banks & Thompson, 1987; Anholt, 1992; Stoks, 2001a). Also, females are more at risk during oviposition as this can take long periods of time during which the female is exposed to predation from both air and water. In other coenagrionids, longevity has also been found to be similar between the

sexes (Robinson *et al.*, 1983; Banks & Thompson, 1985b; Hamilton & Montgomerie, 1989; Anholt, 1997).

Table 5.5. Estimated daily survival rates (φ) for damselfly species from field experiments reported in the scientific literature. Method of calculation is shown where possible. Methods shown in capitals refer to the program used to select an optimal model derived from the CJS based on AIC. Sources marked * were taken from **. Where more than one value was reported the greatest is given.

Species	♂ φ	♀ φ	Method	Source
Coenagrion mercuriale	0.85	0.85	MSURGE	Chapter 5 Beaulieu
Coenagrion mercuriale	0.78		MSURGE	Chapter 5 Itchen
Ischnura pumilio	0.82	0.84	MSURGE	Chapter 4 Latchmoor
Ischnura pumilio	0.94		MSURGE	Chapter 4 Cornwall
Ischnura elegans	0.81	0.58	MARK	Anholt *et al.* (2001)
Lestes disjunctus	0.77	0.83	SURGE	Anholt (1997)
Pyrrhosoma nymphula	0.87	0.86	Jolly	Bennett & Mill (1995)
Hetaerina cruentata	0.98		Jolly	Cordoba-Aguilar (1994)**
Ischnura denticollis	0.71		Manly-Parr	Cordoba-Aguilar (1993)
Calopteryx aequabilis	0.77			Conrad & Herman (1990)*
Calopteryx virgo	0.86			Cordero Rivera (1989)*
Calopteryx haemorhroidalis	0.94			Cordero Rivera (1989)*
Calopteryx xanthostoma	0.66			Cordero Rivera (1989)*
Mnais pruinosa	0.94			Nomakuchi *et al.* (1988)*
Ischnura gemina	0.93		Jolly	Hafernik & Garrison (1986)
Argia sedula	0.79		Jolly-Seber	Robinson *et al.*(1983)
Mnais pruinosa	0.94			Higashi & Ueda (1982)*
Ischnura gemina	0.95	0.90	Jolly	Garrison & Hafernik (1981)

The calculated daily survival values were very similar to those for mature *I. pumilio* (Latchmoor male mean = 0.82; Latchmoor female mean = 0.84; Cornish overall estimate = 0.88; see Chapter 4). A summary of published mean daily survival rates for adult zygopterans is presented in Table 5.5. In a study of *Hetaerina cruentata,* Cordoba-Aguilar (1994) claims to report the highest survival rate for the Calopterygidae and possibly the Odonata. However, this study is one of many included in Table 5.5 that estimated mean daily survival using values which are greater than 1, which are biologically impossible. Those parameters estimated on a boundary or with 95% confidence intervals exceeding the boundary have been omitted in this analysis to give a mean of reliable estimates only. In general the survival rates estimated in this study for *C. mercuriale* are among the highest of those estimated for other coenagrionids and damselflies in general (see Table 5.5).

Sex also had a significant effect on recapture rates, with males being over three times as likely to be captured as females on average. This finding is well supported by other results reported in this thesis and in published studies (e.g. Parr & Parr, 1972; Waage, 1972; Garrison, 1978; Van Noordwijk, 1978; Bennett & Mill, 1995; Anholt, 1997; Cordero Rivera & Andres, 2001), and suggested reasons are discussed above and in Chapter 4.

The probability of recapturing individuals also varied between sites and stochastically through time. The interactive nature of these effects indicates that different temporal factors affected different sites to some extent. Changes in weather variables may have had slightly different effects at different sites, for example reduced captures due to strong wind would be less marked at sheltered sites. It may also reflect variation in surveying effort across sites on different occasions, although effort was standardised as much as possible. Recapture rates as indicated by the average values for each site (males and females) were greatest at Peaked Hill (0.25), followed by Roundhill (0.21), and Crockford and Hatchet Stream had similarly low values (0.14 and 0.13 respectively).

This is likely to be an effect of density as Peaked Hill and Crockford were similarly large areas, but many more individuals were marked at Peaked Hill (5176 compared to 3084 at Crockford). Likewise, Roundhill and Hatchet Stream were relatively small sites, but at Roundhill over 7 times as many individuals were marked (1743 at Roundhill, 241 at Hatchet Stream). Where densities were higher, more time could be spent catching and processing animals, whereas at low densities more time is spent searching in between captures. This may have resulted in the increased capture rates at the higher density sites. At the highest densities, recapture rates can be reduced due to the limit imposed by handling time on numbers processed (see Chapter 4), however these densities may not have been reached in the current study. In addition, although Crockford and Peaked Hill cover similar areas overall, the majority of recaptures are concentrated in one part of the site at Peaked Hill, whereas Crockford had several smaller areas of concentrated recaptures, which again would have resulted in more time spent travelling between areas and therefore less time processing animals.

Some parameters were again estimated on the boundary (close to 0 or 1) in this analysis, as described above for the Itchen data, and this could have introduced bias into the estimates produced from this model. However, overall the analysis of the Beaulieu population is somewhat more reliable, as once the general model had been modified to account for transience and trap dependence it was shown to fit the data well, which was not true of the Itchen data. A ĉ value close to 1 (1.09) for Beaulieu also indicated that there was little residual overdispersion, and the resulting modification of $QAIC_c$ values was less severe, allowing more parameterised models to be selected where appropriate (Cooch & White, 2006). However, aside from the effect of sex on survival rates indicated for the Beaulieu data, for which the effect size was so small as to be considered negligible, the best models selected in both analyses were identical. It was determined that survival rates declined through the season as well as exhibiting some stochastic variation through time due to weather and could be quite different between sites, although not all differed. Recapture rates also varied stochastically through time due to weather conditions and differed between sites. Recapture rates were found to be approximately three times lower in females than in males overall for *C. mercuriale* in this study.

The estimated daily survival rates for *C. mercuriale* were mostly very similar to those estimated for *I. pumilio* (Chapter 4). They were also subject to variation caused by similar factors, declining as the season progressed and varying through time due to weather. Increased survival rates in mature females were detected in both species. However, as discussed above and in Chapter 4, these differences were very small and may not be considered biologically meaningful. In order to establish whether an effect of sex on survival rates truly exists, future studies should carefully consider the areas included in the study, in order to ensure habitat where females roost and feed are included, to ensure that they are better represented in the data.

5.5.4. Population size

The maximum two-daily population size estimates of 12500 at Itchen and 7300 at Beaulieu are among the highest of those published for damselfly

species. Two-daily population size estimates are likely to be more reliable than total estimates, as described in Chapter 4. The maximum daily estimate for *Ischnura denticollis* at a large artificial pond in Mexico was 1400, which is the highest published daily estimate (Córdoba-Aguilar, 1993). Other studies have reported maximum daily estimates of 150 for *Ischnura gemina* (Garrison & Hafernik, 1981), 700 *Lestes disjunctus* (Anholt, 1997) and approximately 700 *Lestes sponsa* (Stoks, 2001a) using the Manly-Parr method, and 500 male *Argia sedula* using the Jolly-Seber method (Robinson *et al.*, 1983). Average daily population size estimates are generally much smaller than these values, as in most cases the population exhibits at least one marked peak in numbers during the study period. This is also the case in the *C. mercuriale* populations studied here, where mean population size was similar in both sites (Mean ± s.e.: Itchen: = 4700 ± 766; Beaulieu = 4300 ± 471). However, these estimates suggest that although the distribution of *C. mercuriale* is decreasing in the UK, where populations exist they can be very large.

As no movement was recorded between the sub-sites in both study sites, individual abundance estimates are perhaps more relevant from a conservation viewpoint. The smallest of the *C. mercuriale* populations studied here were Highbridge, Itchen (maximum daily estimate 780, mean = 494 ± 67.6) and Hatchet Stream, Beaulieu (maximum daily estimate 320, mean = 144 ± 24.4). These values are small in comparison to the other sites studied here but were larger than those for *I. pumilio* in the Cornish population (maximum daily estimate 100, Chapter 4) and *I. gemina* (Garrison & Hafernik, 1981), both of which are considered to be less threatened than *C. mercuriale*.

The calculated total population size estimate for the whole Beaulieu population (44722 ± 1621) was somewhat higher than that calculated by Thompson & Watts (2004). Their estimate (39913) was produced using Jolly-Seber (JS) estimates of daily population size for males, which were then doubled to account for females. These daily censuses were then summed and divided by average lifespan calculated as mean number of days between first and last capture. It was determined in Chapter 4 that the JS

method consistently underestimated population size in *I. pumilio* and *C. mercuriale*, and this seems to be supported here. However these estimates are reasonably close and given the computational requirements of HT estimates, JS methods may be preferred given that both methods are likely to violate assumptions and thus contain substantial error.

Table 5.6. Sex ratio of *C. mercuriale* at Itchen and Beaulieu, and *I. pumilio* at Latchmoor and Cornwall for comparison. Observed sex ratio is calculated from numbers captured and marked. HT mean is the mean sex ratio across two-day capture occasions as calculated using the Horvitz Thompson method. HT total is the ratio of the total male and female populations calculated using the method described in Chapter 4. All values represent the ratio of males to one female.

Site	Observed	HT Mean	HT Total
C. mercuriale Itchen	7.3	4.6	2.6
C. mercuriale Beaulieu	4.3	2.5	1.5
I. pumilio Latchmoor	1.2	0.88	0.54
I. pumilio Cornwall	1.8	2.0	1.5

Horvitz-Thompson estimates of population size are strongly male biased in both populations (Figs. 5.5 & 5.6 and Table 5.5). The potential reasons for such a skewed sex ratio include higher female mortality during the immature phase, sex ratio bias at emergence, and lower recapture and survival rates in females. Calculated sex ratios are presented in Table 5.3, and are male biased in all cases, more so in the Itchen population. This is likely to have been at least partly caused by the large proportion of females omitted from the analysis by sampling design. Searches were performed along linear sections of stream in the Itchen study area, as opposed to the wider searching techniques employed at Beaulieu. This was partly due to the nature of the habitat in the two areas: Itchen comprised linear stretches of stream, with varying accessibility, whereas the Beaulieu site covered a complex network of wet and dry areas. Neither study specifically aimed to cover areas of water and hinterland equally, as did the *I. pumilio* study at Latchmoor, but the nature of the habitat at Beaulieu was more conducive to including at least some of the areas surrounding water. As females spend significantly more time away from the water than males, it is inevitable that a proportion of females will have been missed by employing these searching strategies. Foster & Soluk (2006) found significant differences in habitat use between male and female *Somatochlora hineana* and suggested that this is

often the cause of observed male biased sex ratios in MRR studies (see Chapter 3).

Sex ratios calculated for the Beaulieu population were closer to those reported for the Cornish *I. pumilio* population (Chapter 4) than to the Itchen values, although the ratio of observed males to females was still considerably higher, which was due to the different search methods (Table 5.6). The mean estimated two-daily sex ratio was male biased in the Beaulieu (2.5 : 1) and Itchen (4.6 : 1) populations. This may be considered the more reliable estimate as it takes into account recapture rates which are often said to be the cause of bias in sex ratios in MRR studies of odonates, but does not suffer the problems of the HT total method outlined in Chapter 4.

A review of sex ratio in odonates at emergence undertaken by Corbet (1998) indicated that the proportion of males was greater than unity in 65% of studies (fitting certain criteria and with n>299) based on collected zygopteran exuviae. The study attributes this skew to differential survival of eggs and/or larvae and differential predation of larvae. However, the maximum skew reported was 55% males, which cannot account for any of the *C. mercuriale* sex ratios reported in this thesis. As exuviae were not collected as part of this study, the sex ratio at emergence could not be established, but a previous study of emerging *C. mercuriale* found that the sex ratio was significantly male biased at 1.35 : 1 (Purse & Thompson, 2003b). Interestingly, the only study in this thesis to attempt to include all areas used by females (Latchmoor) found a female biased sex ratio based on mean daily HT estimates. This suggests that the male biased sex ratios reported in most other studies may be at least partly due to underrepresentation of females in the data.

In *Sympetrum danae*, female biased sex ratios at emergence were found to switch to an excess of males at maturity (Michiels & Dhondt, 1989). Cordoba-Aguilar (1993) recorded a sex ratio of approximately 2:1 males to females in mature adult *I. denticollis*, but found that sex ratio did not differ significantly from unity when tenerals only were considered, indicating that

the observed skew in adult numbers was attributable to differential mortality during maturation. Females may experience greater mortality during the immature stage which is often excluded from MRR studies, including those analysed in this chapter. Reduced female survival during the pre-reproductive stage has been reported in several indirect studies of survival rate (Anholt, 1991; 1997), but studies using population modelling methods such as those used here have found the converse (Cordero, 1994; Anholt *et al.*, 2001) including the study of *I. pumilio* discussed in Chapter 4. Female odonates may experience increased pre-maturation mortality compared to males (Banks & Thompson, 1985a; Bennett & Mill, 1995; Stoks, 2001b), as the length of the immature period is considerably longer in females (Cham, 1993; Corbet, 1999). This may result in reduced female numbers even where daily mortality is equal between the sexes as it is acting across more days.

Many examples of a male biased sex ratio in marked individuals exist in the literature (e.g. Robinson *et al.*, 1983; Banks & Thompson, 1985b; Córdoba-Aguilar, 1994; Cordero Rivera & Andrés, 1999) although few consider recapture probabilities explicitly in their calculations (but see Anholt, 1997). However, many claim that low recaptures rates in females due to differences in behaviour, colouration and habitat use cause male bias in sex ratio, and that the true ratio is unity. Stoks (2001a) conducted a test of the commonly suggested hypotheses for male biased sex ratios, using MRR data on *Lestes sponsa* which was analysed using both number of captures and with Manly-Parr population size estimates, and found that the true population sex ratio was male biased. The study considered the areas surrounding ponds as well as the water's edge and found that the sex ratio in those areas was in fact female biased, but not sufficiently so to compensate for the male bias observed at water. Reduced encounter of females due to cryptic behaviour or colouration was shown not to be the cause of the bias, as observations of a semi-natural population (where capture probability was said to be 100%) supported findings from the natural population. The interval between visits to the water was longer in female *L. sponsa* than males, and as females only come to the water to oviposit, they are less likely to be captured the following day as new eggs must be matured. This may be interpreted as

trap-shyness, as females caught one day are less likely to be caught the next. This difference in capture probability between females can result in negatively biased capture probabilities (Kendall *et al.*, 1997), which will overestimate female population size. Therefore, reduced frequency of female visits to water cannot explain male biased sex ratios calculated using MRR modelling methods (Stoks, 2001a). This comprehensive study of sex ratio concluded that the observed male biased sex ratio was real in *L. sponsa,* as spatial distribution, longer intervals between visits to water in females, or their increased cryptic behaviour and colour could not explain the observed bias.

Sex ratio can determine which sex competes for mates and the intensity of that competition. Furthermore, the probability of finding a mate and the effective population size may both be influenced by sex ratio (Garrison & Hafernik, 1981; Fincke, 1982; Stoks, 2001a). In order to establish a more reliable estimate of the true sex ratio in adult *C. mercuriale* populations, an MRR study encompassing all areas of habitat utilised by the species should be undertaken, and a Horvitz-Thompson estimate of daily population size calculated. The methodological and modelling problems encountered in this study prevent a reliable and conclusive estimate to be made using these data.

5.5.5. Summary

Coenagrion mercuriale inhabits increasingly restricted areas in the UK and this is largely due to its specialised habitat requirements and declining availability of that habitat (Rouquette & Thompson, 2005). The species has undergone a 30% reduction in UK distribution since 1960 and great efforts are now being made to conserve the species (Watts *et al.*, 2004). This study has shown that even within a relatively small group of sites, survival rates can differ considerably. In order to conserve the species where it persists, efforts should be made to manage the habitat at sites where survival rates are decreased, according to sound management guidelines (e.g. Thompson *et al.*, 2003; e.g. Rouquette & Thompson, 2005). In addition, where inbreeding and low levels of genetic variation are indicated and may be the cause of reduced survival, population augmentation by reintroduction may

be considered in order to rectify this. Again this should be given careful consideration as isolated populations can be locally adapted (Watts *et al.*, 2004). However, small population size and low levels of dispersal such as that reported at Mariner's Meadow in the Itchen study (Rouquette & Thompson, 2007a), along with reduced survival rates suggests that the population is in danger of extinction and some management should be undertaken to prevent this.

The results of this study have implications for future MRR study design confirming those discussed in the previous Chapter. The data analysed here caused several structural problems when fitting standard MRR umbrella models, and this has inevitably led to bias in the results. Because of the highly specific requirements for a data set to be suitable for MRR analysis using sophisticated software such as MSURGE, it is suggested that data collected for other purposes may be less suitable due to problems with lack of fit. By using a data collection methodology designed to minimise structural failure, MRR modelling is more likely to give reliable results. This is especially pertinent when the results are required to make conservation management decisions, as incorrect judgements can have irreversible effects. Survival rates obtained from such analyses can be highly useful in population viability analysis and modelling potential outcomes of management programs (e.g. Pryde *et al.*, 2005). Analyses of this kind using the rates obtained in this study may be useful in initial planning and predictive modelling; however, more robust estimates should be obtained before using them for management purposes. However, these are the first estimates of survival and its influencing factors in *C. mercuriale*, and provide a basis for further investigation. Specifically, clarification of the differences in survival and recapture rates between sexes and a more comprehensive investigation of its effect on sex ratio, would allow reliable estimates of population vital rates and the size of the groups they effect to be used in modelling real conservation initiatives.

Chapter 6: Conclusion

Each of the preceding chapters contains a full discussion of the results therein. As such, this chapter will consider the main conclusions of the thesis as a whole and the potential conservation management outcomes. It will discuss implications for study design and monitoring and make some suggestions for future work on *Ischnura pumilio*. The contribution to existing knowledge of *Coenagrion mercuriale* will also be discussed.

6.1 *Ischnura pumilio* in south-west England

Little was previously known of the status and distribution of *I. pumilio* in the UK and very few quantitative studies of any aspect of the species' biology have been published. This thesis has therefore raised the level of ecological knowledge about *I. pumilio* considerably. It includes the first large scale MRR studies of the species, the first quantitative assessment of its movement potential, the first attempt to describe quantitatively its habitat requirements and the first estimates of population size.

The site at Latchmoor, New Forest, was chosen as the focus for the first year of sampling due to its well-established status. The population at Latchmoor was estimated at *c.*8700 and the habitat was among the best surveyed based on previous notions and the results presented here. As such it may be one of the most important sites for the species in the UK and is certainly thought to be one of the largest. Of the other sites surveyed, only a few had populations of *I. pumilio* comparable to that at Latchmoor based on the single-day survey data collected in 2006. Great Wheal Seton (estimated at *c.*500, single-day count = 26) and potentially Stepper Point (single-day count = 53) had the largest populations in Cornwall, where habitats are highly variable in type, quality and size. The largest populations in Devon were thought to be at Cadover Bridge (single-day count = 51) and

Smallhanger Waste (single-day count = 29), though no population size estimates were made at these sites. Devon habitats were generally more uniform in their type and size, and given the large areas of potential habitat in Devon it is likely that several more large populations exist, which is also the case in the New Forest. Many of the previous *I. pumilio* sites surveyed had no current population, which may be due to a process of periodic colonisation and extinction events or may be a result of vegetation encroachment. The latter is certainly likely in several cases. Populations of *I. pumilio* in this study were generally much smaller than those of *C. mercuriale*, even Latchmoor which is thought to be an *I. pumilio* stronghold. This suggests that *I. pumilio* may be at even greater risk of inbreeding than *C. mercuriale*.

Very few published studies of *I. pumilio* in the rest of Europe exist, but the species reportedly has a more continuous distribution across mainland Europe and into North Africa (Askew, 1988; Dijkstra & Lewington, 2006). It has been recorded in the Azores, an archipelago of volcanic islands isolated from the European coast by approximately 1500 km (Cordero Rivera *et al.*, 2005) and is also widespread in the mountains of Middle Asia, where it occurs from the plains to the alpine areas up to 3360m above sea level (Borisov, 2006). It is likely that the populations across its mainland distribution are stronger, larger and better connected than most of those in the UK. As such, the threats to *I. pumilio* from habitat fragmentation in the UK make its conservation here a priority in comparison to the rest of its global range.

It is not the purpose of this thesis to advocate the allocation of resources to conserving species which are at the edge of their global range and as such only nationally rare. However, the persistence of high profile, charismatic species has clear educational benefits at a much wider scale than the single species considered, and has popular and political support (Hunter, 2002). Furthermore, the conservation of such species will no doubt benefit countless other species which depend on the declining habitats that must be

maintained in order to achieve this; species which by association are likely to also be in decline (Thomas, 1994).

Ischnura pumilio inhabits early-successional and highly disturbed habitat which may have been common in Britain historically. Thomas (1994) suggested that sedentary species such as *I. pumilio* colonised Britain between 5000 and 8000 years ago when temperatures were warmer and inhabited different and more widespread niches. These species may then have survived due to the development of silviculture and agriculture, which cleared wild woodland and maintained a level of suitable habitat. Traditional land management would have favoured sedentary species, as new, early-successional areas were created frequently and within small distances of previous areas. It was then suggested that the relatively recent expansion of the human population and associated, accelerated habitat loss has resulted in these species becoming 'trapped' by an increasingly fragmented habitat. Thomas noted that although species occupying early-successional, ephemeral habitats should be highly dispersive in order to colonise newly formed habitat effectively (Southwood, 1977), studies of such species commonly find them to be highly sedentary.

Many of the modern sites occupied by *I. pumilio* in the UK are artificial, man-made wetlands, which are either still in use as mineral extraction sites or similar, or have recently ceased to be so. Once extraction processes have ceased, colonisation by early-successional plant species progresses rapidly without maintenance, and *I. pumilio* is soon excluded. It is therefore important to ensure that vegetation is cleared or new areas of suitable wetland are created nearby for dispersing individuals to colonise (Fox *et al.*, 1992). An alternative to this is to allow the land to be grazed by livestock, which creates poaching of the water's edge and prevents succession from progressing too far. *Ischnura pumilio* therefore poses a conservation challenge, as its habitat may need management on a rotational basis to provide some degree of bare substrate with restricted vegetation (Fox & Cham, 1994).

Despite the strength of some UK populations such as Latchmoor, the persistence of *I. pumilio* still requires a degree of conservation management. Populations are isolated given the range of movements observed in this study, and without dispersal to augment genetic diversity even those strong populations are at risk of developing high levels of inbreeding. Given the predicted change in climatic conditions (Government figures reported in 2008 suggest a temperature rise of 4° this century) and associated change in habitat quality and availability, populations without sufficient levels of genetic variation are unlikely to be able to adapt and thus face extinction (Dieckmann *et al.*, 1999). The estimated size of the Great Wheal Seton population is much smaller than those found to have high levels of inbreeding in *C. mercuriale,* and as such is likely to suffer the same effects. Combined with the species' apparently limited dispersal capability, and therefore ability to move to newly formed suitable habitat, the future of *I. pumilio* in the UK is uncertain at best.

The species does have some advantages over others under changing environmental conditions. It is able to tolerate high levels of pollution and human disturbance (Chapter 3) and to exist in such ephemeral niches as tractor tyre ruts (Hammond, 1983; Cham, 1996) and motorway storm retention pools (Scher & Thièry, 2005). Therefore, it is well equipped to deal with the inevitable increase in human activity given the rapid expansion populations are undergoing globally, and the associated levels of pollution and landscape modification. *Ischnura pumilio* has been recorded in agricultural landscapes, particularly those associated with large livestock (Fox, 1987; 1989), and management of water bodies in such areas to favour the species may be one way to secure its persistence.

The ability of *I. pumilio* to colonise newly formed habitat is still under question. Previous reports have suggested it is a long-range disperser which can rapidly colonise newly formed habitat (Askew, 1988; Fox, 1989; Cham, 1996). However, although the mechanism of upward flight and dispersal in the boundary layer has been proposed (Fox, 1989; Cham, 1993), no long-range dispersal movements have been recorded in this study or otherwise.

Furthermore, recording of colonisation events is rare and inherently difficult. To obtain a good overview of the metapopulation structure of this species would require an exceptionally high level of monitoring, given the potential brevity of site occupancy (1 year where univoltine) and range of habitats potentially occupied. Historically, this difficulty of recording may have led to vast underestimation of the species range in the UK, and further problems of identification can only have exacerbated this (Fraser, 1941; Cotton, 1981). Given the species ability to inhabit small, ephemeral sites and those which appear highly unsuitable for other odonates, there may be numerous small populations in the UK, particularly the south and west, where *I. pumilio* exists in a yearly changing configuration of ephemeral patches. If this were the case, the need for long-range dispersal would be reduced, as individuals would need to travel only short distances to find a "stepping-stone" patch of habitat. This implies significant difficulties for future monitoring of the species, as by nature these sites will be very difficult to identify. However, it is encouraging for the species' persistence.

The lack of observations of the upward flight behaviour described previously (Fox, 1989; Cham, 1993) has thrown some doubt on this as a potential dispersal mechanism. However, the two populations where MRR studies were carried out were strongholds of the respective regional *I. pumilio* populations, and as such may have lacked the cues necessary for dispersal. Furthermore, given the absence of any other sites in the immediate local area (nearest known population to Latchmoor was Shipton Bottom at 26km away, nearest to Great Wheal Seton was Chapel Porth 2 at 9km) the genes for dispersal may have been lost in these well-established populations (Dumont & Verschuren, 1991; Dieckmann *et al.*, 1999; Watts *et al.*, 2004).

This thesis has unfortunately done little to resolve the apparent dichotomy of *I. pumilio*'s dispersal behaviour, as the species was found to have similar movement characteristics to other similarly sized odonates, and no upward flight was observed. This highlights the difficulty in studying the long-range movement potential of such species, as large study areas must be intensively

monitored if a true picture is be obtained. Although not observed, long-range dispersal clearly occurs in *I. pumilio* as the species occurs on several islands in the Azores, which are approximately 1500km from the European mainland (Cordero Rivera *et al.*, 2005). The species was first recorded there in 1933 (Navas, 1933, as cited in Cordero Rivera, 2005) and is likely to have persisted since then, given the low probability of repeated colonisation events. However, the species did colonise those islands at some point and carriage on wind currents seems the most likely mechanism. This suggests that *I. pumilio* movement occurs at two distinct spatial scales. On a large scale, the reputation of the species to colonise distant sites and its clear ability to disperse many hundreds of miles across ocean, gives weight to the dispersal by wind hypothesis. Most of the movement recorded in this study was short-range; individuals were shown to be stronger fliers than previously thought, being unaffected by wind direction and present on days with weather precluding activity in most other species (Chapter 2). However, medium-range movements were absent from the study, either due to study size being too small to record it, or because it is rare or absent, at least in high quality habitat.

A similar situation exists with *Pantala flavescens*, which is notorious for strong, vigorous flight and long-range dispersal away from natal habitat assisted by wind. However, populations of the species on Easter Island, one of the most isolated places on Earth, exhibit extremely different movement patterns. Animals were observed to glide and hover at low altitudes and to fly so weakly as to be almost catchable by hand (Dumont & Verschuren, 1991). The authors suggested that poor larval nutrition led to reduced dispersal behaviour in early generations following colonisation and that this was subsequently favoured by selection, as individuals dispersing from such an isolated place must suffer almost 100% mortality. A later study (Samways & Osborn, 1998) revealed that the island population had smaller and more asymmetric hind wings than mainland populations of *P. flavescens*, further implicating genetic mechanisms for weaker flight in the form of inbreeding and loss of genetic diversity.

The same process may well have occurred in *I. pumilio*, although the spatial scale of both its movement capability and habitat isolation are smaller. In good-quality but isolated habitat, the tendency to disperse is a clear disadvantage and so may be selected against. Furthermore, these isolated populations may be suffering from a loss of genetic diversity, which may result in behavioural or morphometric changes leading to reduced dispersal. What this study may not have observed is the converse situation, where habitat is poor or ephemeral but new areas are created nearby, dispersal may be more common and upward flight behaviour may occur and be observable. This demonstrates that there are no absolute answers where dispersal is concerned, and that there is a right and a wrong time to disperse.

6.2 Management

Previous notions of highly specific habitat requirements in *I. pumilio* (Fox, 1987; Fox, 1989; Cham, 1990; Cham, 1991; Cham, 1992a; Fox & Cham, 1994; Daguet, 2005) were not fully supported by the findings of this thesis. The species was found at a range of water depths and pH levels, at polluted sites and at sites with widely varying management regimes, grazing and disturbance levels. However, as previously suggested the sites surveyed were generally slow-flowing, with some bare ground in and around the water's edge and relatively open in terms of shade from tall vegetation. Application of these findings to conservation projects, particularly at lower latitudes, would require validation of the model by application to data sets from other regions (Guisan *et al.*, 2002). However, as the model was developed at sites where *I. pumilio* was known to have previously existed, the findings strongly suggest features which are important for *I. pumilio*'s persistence at existing sites.

Management of areas away from water should certainly be incorporated into any habitat management plan, as different individuals may be present and may exhibit different behaviours (Chapter 3; Foster & Soluk, 2006). The width of an effective buffer will depend on whether management is to be

directed at *I. pumilio* alone or at a species assemblage. This study indicates that a buffer of 25m would exclude only 5.8% of *I. pumilio* individuals and may be sufficient for the necessary roosting, mating and feeding requirements of the species However, at sites with lower habitat quality individuals may have to fly further to roost, so it must be ensured that areas for roosting are maintained within a reasonable distance given the species movement capacity.

Ischnura pumilio was more frequent at sites with low counts of other odonate species in this study. This may make management of sites for the species difficult if other species are also to be considered. Its requirement for disturbance puts *I. pumilio* at odds with other odonates that might need habitat management, and may be the reason for the observed low species counts at *I. pumilio* sites. There are also implications for the monitoring of *I. pumilio*, as records submitted to the British Dragonfly Society are largely generated by volunteers who may prefer to visit sites with high species counts. Corbet (1960) suggested that *I. pumilio*'s preference for early-successional, disturbed habitat compensates for its low competitive ability. This has not been explicitly considered in this thesis, but is supported by reduced odonate counts at key sites. As *I. pumilio* is the smallest British odonate, is relatively sedentary and was observed to be predated by *Libellula depressa* during this study; it is likely that it is commonly preyed upon by larger odonates. Along with certain predation of larvae by other, larger odonates, this may be enough to exclude the species from sites with many anisopteran species. However, *C. mercuriale* adults also require a level of disturbance which maintains early-successional habitat (Rouquette & Thompson, 2005), therefore the conservation of these two rare species may be complementary. This is supported by their co-occurrence in 5 of the sites surveyed in this study.

6.3 Study design

The importance of the inclusion of hinterland in studies of *I. pumilio* was highlighted in Chapter 3. Females were shown to spend more time there

than males and a significant proportion of matings were recorded away from water also. The inclusion of an area larger than that used by *I. pumilio* in the MRR study at Latchmoor resulted in an almost even estimated daily sex ratio and greater confidence in estimates of other female traits.

Individuals were generally present later in the day than other well studied damselflies (typically peak hours were between 11am and 6pm), and study design should consider this, particularly if other odonates are to be surveyed concurrently.

The indicated high mortality of immature males may have been due to the criteria used to define this stage in males unintentionally including part of the teneral phase. Immature males were defined as not having developed the characteristic blue tail, but past the stage of shiny wings that clearly indicates an individual is newly emerged. However, during this phase the exoskeleton and wings may not have been completely hardened and marking or handling may have caused damage. This highlights the difficulty of estimating ecological parameters in damselflies during the pre-maturation period, which can have important implications for studies of dispersal and sex ratio. Dispersal during the maiden flight has been observed in *Coenagrion puella* and is believed to be the principal dispersive phase for some species (Anholt, 1990; Corbet, 1999). However, this behaviour was not observed in *I. pumilio* in this study, or in *C. mercuriale* during the Itchen study. Studies of behaviour, sex ratio and survival during the phase between emergence and maturity require tenerals to be marked, but this may confound the study by directly influencing behaviour and survival due to damage to the wings or other morphology. Marking with stable isotopes has been used to assess dispersal in stoneflies (Briers *et al.*, 2004) but requires addition of nutrients to water, which may disturb the balance of some water bodies leading to changes in habitat quality. Furthermore, these methods are not permitted for protected species such as *C. mercuriale* and therefore cannot be recommended for *I. pumilio* either. Until a non-destructive method of marking newly emerged damselflies can be found, it seems some biological questions will remain unanswered.

Surveying for this thesis took place during two summers which were, fortunately, highly favourable in terms of weather. However, the two subsequent summers have been significantly cooler and windier, and have seen flooding across parts of the UK. As such, populations may have significantly declined due to inclement weather preventing foraging and breeding. A monitoring program to establish the current status of these and other key odonate sites across the UK would therefore be beneficial. Monitoring of adult *I. pumilio* at existing sites may be successfully achieved with relatively little effort. Transect walks were shown to be correlated with Horvitz-Thompson (HT) estimates of population size in this study, and as such represent a useful method for large-scale monitoring. This can be undertaken by members of the general public and used as a conservation management tool on a national level if carefully administered. Further work should be done to establish a relationship across sites and years so that a predictive model of true population size can be developed. Furthermore, comparisons of HT and other methods requiring MRR data revealed that Jolly-Seber estimates produced using simple software are similar to those produced using complex methods such as HT estimation. Although serious violations of the underlying assumptions of the JS method must be accepted for use of this method, this is true to some degree with any MRR modelling technique, and may be acceptable where time or computational power are limited.

Where complex MRR modelling techniques are to be used, it is important that methodology is designed to minimise structural failure, if reliable results are to be obtained. This is especially pertinent when the results are required to make conservation management decisions. Survival rates obtained from such analyses can be useful in population viability analysis and modelling potential outcomes of management programs. Analyses of this kind using the rates obtained in this study may be useful in initial planning and predictive modelling, although cautious interpretation is advised given the problems with lack of fit previously discussed.

Populations of endangered vertebrates are commonly augmented using captive breeding and reintroduction programs and this has occasionally been attempted in insects also (Pyle *et al.*, 1981). A reintroduction program of *Coenagrion mercuriale* is planned for at least one site in south England and if successful may be extended to more sites. Such programs require careful monitoring and management to ensure conditions remain suitable for the species in question and fluctuations in abundance are documented. Reintroduction of *I. pumilio* may be less straightforward, however, given its occupancy of widely varying habitat types and tendency to remain there for only a few years in all but the best sites. Of those sites supporting long-term, relatively large populations in this study, no clear unifying features exist other than shallow, slow-flowing water and maintained openness of vegetation. Therefore, choosing sites for reintroduction would be difficult and the population may not persist more than a few years without continued management. Additionally, potential source populations would need to be of a sufficiently large size in order to survive the removal of individuals, and have sufficient levels of genetic diversity to adapt to the new habitat. Given the small size of most of the populations surveyed here, these source populations would be hard to find. Alternatively, captive breeding may be employed to provide individuals for reintroduction. Captive anisopterans will not mate or oviposit naturally in captivity, but consistently attempt to escape (Samways, 1993), although more success has been had with zygopteran laboratory populations (e.g. Cordero, 1990).

6.4 *Coenagrion mercuriale*

A significant amount of research into the habitat requirements, dispersal potential and genetic diversity of *C. mercuriale* has been conducted and the status of the species in the UK is relatively well known. The species has been shown to be highly sedentary and to have specific habitat requirements. Certain isolated populations have remarkably low levels of genetic diversity and are a cause for serious concern from a conservation perspective. It was not the intention of this thesis to review the literature

concerning the species, but to add to the body of existing knowledge by reporting survival and population size estimates obtained using the MRR techniques applied to *I. pumilio.* This involved the analysis of two large data sets collected previously for different purposes during the summers of 2001 in Itchen, Hampshire and 2002 in Beaulieu, New Forest.

Coenagrion mercuriale is a conservation priority among European odonates, is listed as rare in the British Red Data Book of Insects (Shirt, 1987), and until 2008 was the only odonate given priority status in the UK Biodiversity Action Plan (HMSO, 1994; 1995). It has a severely restricted range in the UK, occurring at only a few south-western sites (Thompson & Watts, 2006; Watts *et al.*, 2007b). *C. mercuriale* has been more comprehensively studied in the UK than in other European countries (Rouquette & Thompson, 2007a), and estimates suggest that the UK population has declined by 30% since 1960, principally due to anthropogenic factors (Watts *et al.*, 2004). Several mark recapture studies have been undertaken (Purse *et al.*, 2003; Thompson & Watts, 2004; Watts *et al.*, 2004), however, none has made use of the MRR software available to produce estimates of survival and recapture probabilities, or in fact considered survival at all.

This thesis provided the first estimates of survival and recapture probabilities of *C. mercuriale,* in two populations in the south of England. The study revealed that survival rates can vary considerably, even within a small group of sites. In the Itchen study, individuals at Highbridge had higher survival rates than at Mariner's Meadow or LIC, where rates were almost identical. This was attributed to a combination of habitat quality, genetic variation and possibly management. In Beaulieu, the smallest site, Hatchet Stream, had the highest survival rates followed by the two largest sites. As previous genetic analyses found no difference between the Beaulieu sites (Thompson & Watts, 2006), estimated survival differences were attributed to unmeasured factors such as habitat quality or predation. The effect of sex on survival was concluded to be absent or negligible, although the observed and calculated sex ratio was strongly male biased.

This bias may have been due to omission of large numbers of females due to study design, or to real differences in male and female survival which could not be detected for the same reason. The recapture rate of males was three times higher for males than females in this study.

These are the first estimates of survival in *C. mercuriale*, and provide a basis for further investigation. A more comprehensive investigation of sex ratio and survival estimation using data collected with female habitat use in mind would be profitable if survival rates are to be used in modelling real conservation problems. In order to conserve the species in sites where it persists, efforts should be made to manage habitat at sites where survival rates were lower, according to sound management guidelines (Thompson *et al.*, 2003; Rouquette & Thompson, 2005). Sites such as Mariner's Meadow, Itchen which have low levels of dispersal (Rouquette & Thompson, 2007a), small populations and reduced survival rates, may be in danger of extinction and require management to prevent this.

The estimated maximum population sizes during the two studies of *C. mercuriale* in this thesis are among the highest recorded for any damselfly when the study areas were each considered as one population. However, as no movement was recorded between sites in each study, they may be treated as distinct populations (although there was no evidence of genetic isolation at Beaulieu; Thompson & Watts, 2006). The smallest of the *C. mercuriale* populations studied here were Highbridge (maximum two-daily estimate = 780) and Mariner's Meadow (926), Itchen and Hatchet Stream, Beaulieu (320). These values were small compared with the other *C. mercuriale* populations studied but were still larger than the population of *I. pumilio* at Great Wheal Seton (100). This suggests that although the distribution of *C. mercuriale* is decreasing in the UK, where populations exist they can be very large. Furthermore, it highlights the fragile nature of *I. pumilio* populations, as with a seasonal maximum of only 100 individuals, they must be suffering similar levels of inbreeding and loss of genetic diversity as *C. mercuriale*.

Ischnura pumilio is generally considered to be less threatened than *C. mercuriale*. Records of *I. pumilio* exist from over 200 of the 10km UK grid squares used for recording by the National Biodiversity Network (NBN) gateway, compared with only *c.*45 grid squares for *C. mercuriale*. This widespread distribution may indicate greater stability of the UK population. However, the population sizes estimated for the two species in this thesis show that *I. pumilio* exists in much smaller populations that *C. mercuriale*, even when stronghold sites such as Latchmoor are considered. This suggested stronghold of the UK population of *I. pumilio* is considerably smaller than many of the *C. mercuriale* populations estimated. Given that the Beaulieu *C. mercuriale* populations may be considered one metapopulation as they are not genetically distinct, the estimated number of individuals in 2002 (*c.*44700) was vastly different to *I. pumilio* at nearby Latchmoor in 2005 (c. 8700). Small populations can suffer high levels of inbreeding and loss of genetic diversity, as has been shown in marginal *C. mercuriale* populations, which leads to local adaptation and reduced ability to adapt to environmental change. Genetic analysis of *I. pumilio* populations is required to determine their level of connectivity and indicate the levels of genetic diversity present, which will ultimately determine their persistence as climate, and consequently habitat, changes over the next decades. Compared to *C. mercuriale* there have been relatively few published studies of *I. pumilio* (>50 and <30 respectively), most of which were observational studies. It is recommended that greater attention be given to the remaining populations of *I. pumilio* in the UK, as it is unsure how many of those 200+ UK grid squares still hold populations, and the species' conservation status may more similar to that of *C. mercuriale* than previously thought.

6.5 Further work

In order to develop the knowledge of *I. pumilio*'s habitat requirements further, an assessment of the effect of recommended habitat management operations on abundance should be conducted. By doing this now, while the species still has several stronghold populations in the UK, the information

will be usable in the event of a major population decline. Once a decline has occurred, experimental manipulation of habitat becomes much more contentious, and could lead to further extinctions which cannot be rectified if not carefully planned. The habitat preferences of the species have been determined by observational rather than experimental evidence. Experimental manipulation of habitat with follow up monitoring would give additional weight to the findings in this thesis. Monitoring should be carried out over as many consecutive years as possible, to give a good indication of the effect of management and its ability to ensure the persistence of this species.

Limited adult dispersal is common among damselflies (Banks & Thompson, 1985a; Conrad *et al.*, 1999; Rouquette & Thompson, 2007a) and combined with habitat fragmentation suggests that populations may be subject to significant levels of inbreeding across the taxon. Genetic analysis of *C. mercuriale* has revealed that levels of inbreeding in some populations are comparable to those observed in threatened mammals (Watts *et al.*, 2005). Other than those on *C. mercuriale*, there are few published population genetic studies on damselflies, but further work in this area is required if the levels of differentiation between populations and their effects on important ecological characteristics such as dispersal are to be understood. The removal of a leg at first capture was shown to have no effect on survival in *I. pumilio*. This is encouraging for future studies of *I. pumilio* and other odonates, as genetic and behavioural work may be carried out concurrently without the risk of a detrimental effect on survival.

As discussed above, the comparisons of population size estimates and monitoring methods in Chapter 4 gave promising results for the development of a monitoring scheme for *I. pumilio* and damselflies more generally. Mark-release-recapture studies are expensive, time consuming and require a considerable degree of man power if reliable estimates are to be obtained. Nevertheless, in order for a predictive relationship between monitoring counts and sophisticated population size estimates to be established, further MRR work is required to provide concurrent estimates.

However, once this is achieved subsequent work on damselfly species will be greatly facilitated and volunteers will be able to produce highly informative data with minimal training. MRR studies need not be as long as those described in this thesis, but should include sufficient time to mark a reasonable proportion of the population, and should be conducted during good weather when captures can be made every day to avoid problems modelling survival and recapture rates. Ideally, sites from a variety of regions and even countries would be considered over several years for each species, in order to obtain a reliable predictive model.

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Appendix 1

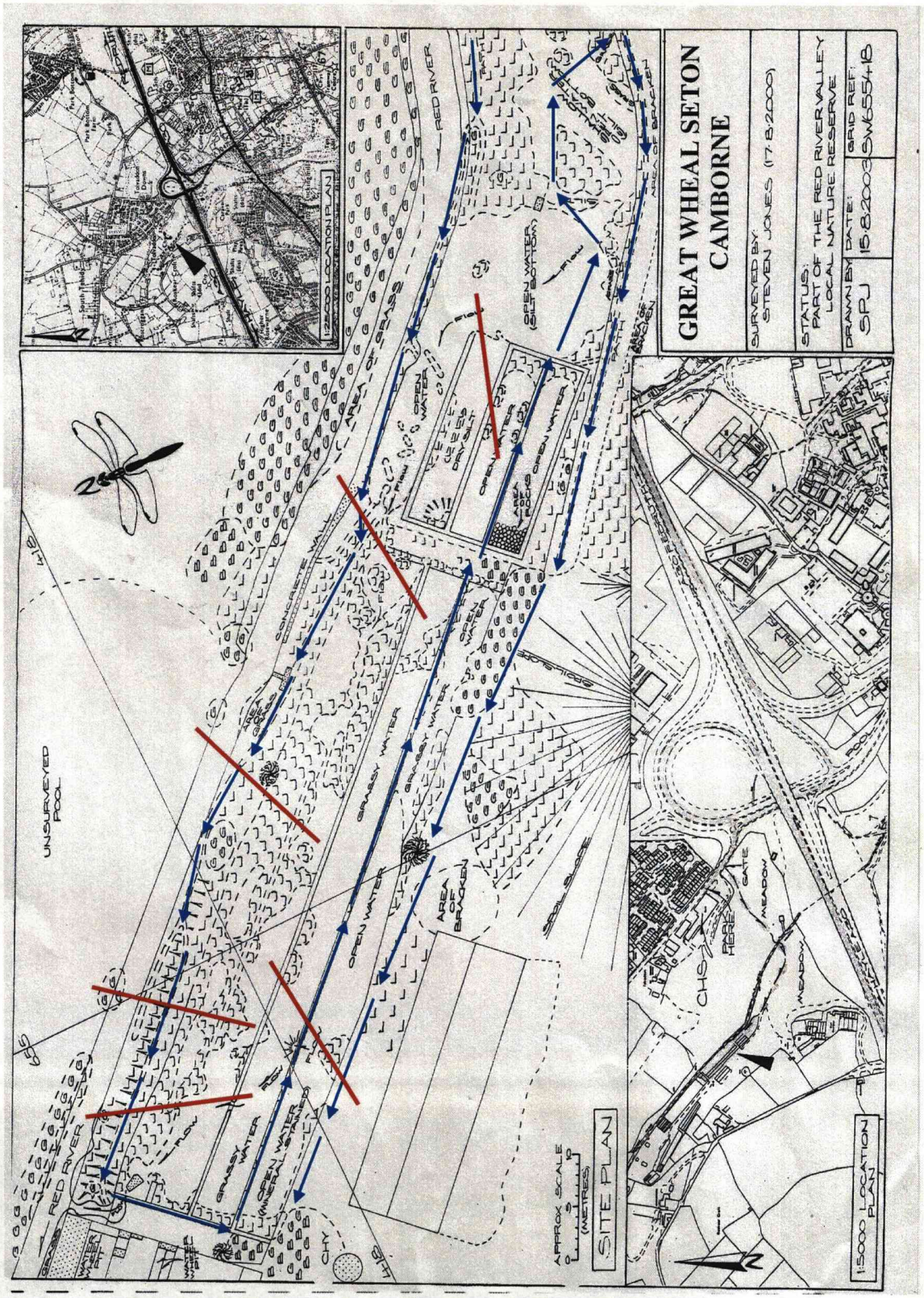

Figure A.1. Map of Great Wheal Seton (site Ae). Transect walk route marked with blue arrows. Site survey transects indicated with red lines; quadrats were placed along these lines starting in the water at 0, 2, 5, 10, and 20m from the start point. Original map drawn by Steve Jones.

Appendix 2

Table A2.1. The complete list of models fitted to the 2005 *I. pumilio* maturity data in order of AIC value. Number of estimable parameters (NP), model deviance and Akaike's information criterion (AIC). Subscripts – a = age (*NL* indicates negative linear effect, 2 indicates two-age-class effect), f = state of maturity on previous occasion, to = state of maturity at current occasion, s = sex, t = time. Terms in square brackets indicate weather effects modelled as constraints on time: *RD* = daily rain, *RF* = rainfall, *MX* = maximum temperature, *MN* = minimum temperature, *CD* = cloud cover, *WD* = wind speed, *RA* = radiation.

Model	NP	Deviance	AIC
$\psi\ f*s\ \varphi\ [a\ NL]+t+f\ p\ t+s$	57	12033.707	12147.707
$\psi\ f*s\ \varphi\ [a\ NL]+t\ p\ t+s$	56	12040.848	12152.848
$\psi\ f\ \varphi\ [a\ NL]+t+f\ p\ t+s$	56	12054.981	12166.981
$\psi\ f\ \varphi\ [a\ NL]+t+f\ p\ to*[t+s]$	81	12005.647	12167.647
$\psi\ f\ \varphi\ [a\ NL]+t\ p\ t+s$	55	12063.141	12173.141
$\psi\ f*s\ \varphi\ a2+t+f\ p\ to*[t+s]$	83	12007.443	12173.443
$\psi\ f\ \varphi\ a+t+f\ p\ to*[t+s]$	100	11979.931	12179.931
$\psi\ f\ \varphi\ a+f+t+s\ p\ to*[t+s]$	101	11977.988	12179.988
$\psi\ f\ \varphi\ a+t+s\ p\ to*[t+s]$	99	11982.047	12180.047
$\psi\ f\ \varphi\ a+t\ p\ to*[t+s]$	99	11982.651	12180.651
$\psi\ f\ \varphi\ a+t+f\ p\ t+s$	75	12031.630	12181.630
$\psi\ f\ \varphi\ a2+t+f\ p\ to*[t+s]$	80	12026.845	12186.845
$\psi\ f\ \varphi\ a2+t\ p\ to*[t+s]$	79	12029.296	12187.296
$\psi\ f\ \varphi\ a2+t+s\ p\ to*[t+s]$	80	12028.513	12188.513
$\psi\ f*s\ \varphi\ a2+t+f\ p\ to*[t+s]$	81	12026.687	12188.687
$\psi\ f*t\ \varphi\ a2+t+f\ p\ to*[t+s]$	99	11992.208	12190.208
$\psi\ f\ \varphi\ a2+t+f\ p\ t+s$	56	12080.007	12192.007
$\psi\ f\ \varphi\ f*[a\ NL+t]\ p\ t+s$	82	12029.426	12193.426
$\psi\ f\ \varphi\ a2+t\ p\ t+s$	55	12087.173	12197.173
$\psi\ f*t*s\ \varphi\ a2+t+f\ p\ to*[t+s]$	115	11968.207	12198.207
$\psi\ f*s\ \varphi\ t+f\ p\ t+s$	56	12087.675	12199.675
$\psi\ f\ \varphi\ a2+t+f\ p\ t*s$	80	12041.012	12201.012
$\psi\ f\ \varphi\ a2+t\ p\ t*s$	79	12048.350	12206.350
$\psi\ f\ \varphi\ a2*t\ p\ to*[t+s]$	103	12001.613	12207.613
$\psi\ f\ \varphi\ a2*t\ p\ t+s$	78	12059.333	12215.333
$\psi\ f\ \varphi\ f*s*[[a\ NL]+t]\ p\ a2+t+s$	123	11970.425	12216.425
$\psi\ f\ \varphi\ f+t\ p\ to+[t*s]$	79	12059.861	12217.861
$\psi\ f\ \varphi\ [f*s]+t\ p\ t+s$	57	12104.854	12218.854
$\psi\ f\ \varphi\ t\ p\ to*[t+s]$	79	12061.267	12219.267
$\psi\ f\ \varphi\ f+t\ p\ t+s$	55	12109.340	12219.340
$\psi\ f*t*s\ \varphi\ a2*t\ p\ to*[t+s]$	140	11940.024	12220.024
$\psi\ f\ \varphi\ a2+[t*RD]+f\ p\ to*[t+s]$	56	12108.373	12220.373
$\psi\ f\ \varphi\ f*s*[[a\ NL]+[t*NL]]\ p\ t+s$	34	12160.036	12228.036
$\psi\ f\ \varphi\ [a\ NL]+[t*MX]+f\ p\ t+s$	32	12164.407	12228.407
$\psi\ f\ \varphi\ f*t\ p\ to*[t+s]$	99	12032.910	12230.910
$\psi\ f\ \varphi\ a2+t+f\ p\ to*t*s$	120	11992.633	12232.633
$\psi\ f\ \varphi\ a2+t\ p\ to*t*s$	119	11995.009	12233.009
$\psi\ f\ \varphi\ [a\ NL]+[t*MX]+f\ p\ to*[t+s]$	59	12115.326	12233.326

ψ f φ t*s p to*[t+s]	104	12025.719	12233.719
ψ f φ a2*f*t p to*[t+s]	142	11950.228	12234.228
ψ f φ a2*t*s p to*[t+s]	148	11939.776	12235.776
ψ f*s φ [a NL]+f p t+s	32	12174.653	12238.653
ψ f φ a+[t*MX]+f p to*[t+s]	76	12093.668	12245.668
ψ f φ f*s*[a NL] p t+s	33	12191.724	12257.724
ψ f φ a2*f*t*s p to*[t+s]	199	11863.789	12261.789
ψ f φ f*t*s p to*[t+s]	139	11991.113	12269.113
ψ f*t*s φ a2*f*t*s p to*t*s	246	11779.132	12271.132
ψ f φ a2*f*t*s p t+s	182	11910.411	12274.411
ψ f*t*s φ a2*f*t*s p f*to*t*s	260	11754.515	12274.515
ψ f φ a2*f*t*s p t*s	204	11869.219	12277.219
ψ f*t*s φ a2*f*t*s p t*s	228	11823.180	12279.180
ψ f φ a2*f*t*s p to+[t*s]	208	11865.980	12281.980
ψ f φ a2*f*t*s p to*t*s	227	11829.159	12283.159
ψ f*s φ a2*f*t*s p to*t*s	226	11832.939	12284.939
ψ f φ a+f p to*[t+s]	82	12123.919	12287.919
ψ f φ a2*f*t*s p [to*t]+s	203	11882.972	12288.972
ψ f φ a p to*[t+s]	81	12127.302	12289.302
ψ f*t φ a2*f*t*s p to*t*s	242	11806.684	12290.684
ψ f φ a2*s p to*[t+s]	57	12191.465	12305.465
ψ f φ a2*f*t*s p [to+s] t	223	11860.393	12306.393
ψ f φ a2+[t*MN]+f p to*[t+s]	57	12193.068	12307.068
ψ f φ a2*f*s p to*[t+s]	60	12188.970	12308.970
ψ f φ a2 p to+[t*s]	55	12200.135	12310.135
ψ f φ a2+f p to+[t*s]	56	12198.520	12310.520
ψ f φ a2*f p to*[t+s]	57	12197.679	12311.679
ψ f φ a2+s p to*[t+s]	56	12199.922	12311.922
ψ f φ i p to*[t+s]	54	12220.731	12328.731
ψ f φ f p to*[t+s]	55	12219.524	12329.524
ψ f φ s p to*[t+s]	55	12220.083	12330.083
ψ f φ f+s p to*[t+s]	56	12219.354	12331.354
ψ f φ f*s p to*[t+s]	57	12219.004	12333.004
ψ f φ a+s p to*[t+s]	81	12192.155	12354.155
ψ s φ a2*f*t*s p to*t*s	250	11861.894	12361.894
ψ i φ a2*f*t*s p to*t*s	246	11870.991	12362.991
ψ t*s φ a2*f*t*s p to*t*s	304	11775.407	12383.407
ψ f φ [a t]+f p to*[t+s]	320	11748.815	12388.815
ψ f*t*s φ a2*f*t*s p to*t	221	11963.203	12405.203
ψ t φ a2*f*t*s p to*t*s	285	11838.863	12408.863
ψ f φ a2*f*t*s p to*t	201	12014.743	12416.743
ψ f*t*s φ a2*f*t*s p t	204	12018.131	12426.131
ψ f φ a2*f*t*s p t	182	12064.357	12428.357
ψ f φ a2+t+f p to*[[t*MX]+s]	35	12391.357	12461.357
ψ f φ [a NL]+t+f p [t*MX]+s	32	12398.174	12462.174
ψ f φ a2+t+f p to*[[t*CD]+s]	35	12522.752	12592.752
ψ f φ [a NL]+t+f p [t*MX+1]+s	32	12638.108	12702.108
ψ f φ [a NL]+t+f p [t*MN]+s	32	12680.553	12744.553
ψ f φ a2+t+f p to*[t*RD+s]	35	12685.045	12755.045
ψ f φ a2+t+f p to*[[t*MN]+s]	35	12685.486	12755.486

$\psi\ f\varphi\ a2*f*t*s p\ to*s$	163	12476.645	12802.645
$\psi\ f\varphi\ a2*f*t*s p\ s$	158	12498.907	12814.907
$\psi\ f\varphi\ a2*f*t*s p\ to+s$	161	12493.646	12815.646
$\psi\ f\varphi\ a2*f*t*s p\ to$	156	12557.636	12869.636
$\psi\ f\varphi\ a2*f*t*s p\ i$	158	12584.048	12900.048
$\psi\ f\varphi\ a2+[t*CD]+f p\ to*[t+s]$	56	12906.862	13018.862
$\psi\ f\varphi\ [a\ NL]+t+f p\ [t*MX]$	31	13004.388	13066.388
$\psi\ f\varphi\ t+s p\ to*[t+s]$	77	12999.232	13153.232
$\psi\ f\varphi\ a2+[t*WD]+f p\ to*[t+s]$	57	15833.856	15947.856
$\psi\ f\varphi\ [a\ NL]+t+f p\ to*[t+s]$	83	15833.856	15999.856
$\psi\ f\varphi\ a2+[t*RA]+f p\ to*[t+s]$	56	15929.455	16041.455
$\psi\ f\varphi\ a2+t+f p\ to*[t*R+s]$	35	16449.230	16519.230
$\psi\ f\varphi\ a2+[t*R\varphi]+f p\ to*[t+s]$	56	17870.851	17982.851

Table A2.2. The complete list of models fitted to the 2005 *I. pumilio* mites data in order of AIC value, Number of estimable parameters (NP), model deviance and AIC. Subscripts – *a* = age (*NL* indicates negative linear effect, 2 indicates two-age-class effect), *f* = mite load on previous occasion, *to* = mite load on current occasion, *s* = sex, *t* = time.

Model	NP	Dev	AIC
$\psi\ f*to+s\ \varphi\ a2*s+t+f p\ s*to+t$	55	8294.847	8404.847
$\psi\ f*to+s\ \varphi\ a2*s+t p\ s*to+t$	53	8300.103	8406.103
$\psi\ f*to+s\ \varphi\ a2+t+f p\ s*to+t$	53	8305.872	8411.872
$\psi\ f*to\ \varphi\ a2*s+t p\ s*to+t$	52	8307.888	8411.888
$\psi\ f*to+s\ \varphi\ a2+t p\ s*to+t$	51	8310.149	8412.149
$\psi\ f*to+s\ \varphi\ a2+f+t+s p\ s*to+t$	54	8305.723	8413.723
$\psi\ f*to+s\ \varphi\ t+f p\ s*to+t$	52	8311.669	8415.669
$\psi\ f+to\ \varphi\ a2*s+t p\ s*to+t$	51	8313.682	8415.682
$\psi\ f*to+s\ \varphi\ a2*f+t p\ s*to+t$	55	8305.97	8415.97
$\psi\ f*to+s\ \varphi\ s+t+f p\ s*to+t$	53	8311.666	8417.666
$\psi\ f*to+s\ \varphi\ a2*s+t+f p\ t+s$	51	8316.766	8418.766
$\psi\ f*to+s\ \varphi\ a2*s+t p\ to+t+s$	51	8317.117	8419.117
$\psi\ f*to+s\ \varphi\ a2*s+t p\ t+s$	49	8322.795	8420.795
$\psi\ f*to+t\ \varphi\ a2*s+t p\ s*to+t$	70	8280.835	8420.835
$\psi\ f+to+t\ \varphi\ a2*s+t p\ s*to+t$	69	8286.617	8424.617
$\psi\ f*to+s\ \varphi\ a2*s+f p\ s*to+t$	37	8351.016	8425.016
$\psi\ f*to+s\ \varphi\ a2+f p\ s*to+t$	35	8358.823	8428.823
$\psi\ s*to+t\ \varphi\ a2*s+t p\ s*to+t$	70	8289.408	8429.408
$\psi\ f*to+s\ \varphi\ a2+s+f p\ s*to+t$	36	8358.012	8430.012
$\psi\ s*to+t\ \varphi\ a2*f*s p\ s*to+t$	59	8320.042	8438.042
$\psi\ f*to+s\ \varphi\ a2*f*s p\ s*to+t$	43	8352.321	8438.321
$\psi\ s*to+t\ \varphi\ a2+f+t+s p\ s*to+t$	71	8296.819	8438.819
$\psi\ s*to+t\ \varphi\ a2*f+t p\ s*to+t$	72	8295.552	8439.552
$\psi\ s*to+t\ \varphi\ f*s+a2 p\ s*to+t$	55	8329.727	8439.727
$\psi\ s*to+t\ \varphi\ s*a2+f p\ s*to+t$	54	8334.089	8442.089
$\psi\ s*to+t\ \varphi\ f*s+t p\ s*to+t$	72	8299.471	8443.471

ψ s*to+t φ a2+f p s*to+t	52	8340.411	8444.411
ψ s*to+t φ a2*f+s p s*to+t	55	8334.583	8444.583
ψ s*to+t φ a2+s p s*to+t	51	8342.922	8444.922
ψ s*to+t φ a2+f+s p s*to+t	53	8340.279	8446.279
ψ s*to+t φ f+s p s*to+t	52	8342.482	8446.482
ψ to+t φ a2*s+t p s*to+t	67	8320.692	8454.692
ψ to+t φ a2*f*s p s*to+t	57	8362.201	8476.201
ψ to+t+s φ a2*f*s p s*to+t	58	8361.479	8477.479
ψ to*t φ a2*f*s p s*to+t	92	8301.452	8485.452
ψ to*t+s φ a2*f*s p s*to+t	93	8300.846	8486.846
ψ to*t φ a2*f*s p to*s	73	8378.03	8524.03
ψ to*t φ a2*s p to*s	66	8395.361	8527.361
ψ to*t φ f*s p to*s	68	8393.318	8529.318
ψ to*t φ a2 p to*t	64	8405.309	8533.309
ψ to*t φ a2*s p t+s	80	8375.114	8535.114
ψ to*t φ a2*f p to*s	68	8399.28	8535.28
ψ to*s φ a2*s p to*t	67	8402.745	8536.745
ψ to*t φ a2*f*s p t+s	88	8364.833	8540.833
ψ to*s φ a2*t p to*t	93	8364.59	8550.59
ψ to*s φ a2 p to*t	65	8423.09	8553.09
ψ to*t φ a2*f*s p t*to+s	126	8301.133	8553.133
ψ to*t*s φ a2*f*s p to*s	130	8293.26	8553.26
ψ to*t φ a2*f*s p to+t+s	90	8374.498	8554.498
ψ to*s φ a2*t*s p to*t	136	8283.798	8555.798
ψ to*t*s φ a2*s p to*s	122	8312.34	8556.34
ψ to*t*s φ a2*s p to*t	172	8220.165	8564.165
ψ t+s φ a2*f*s p s*to+t	56	8452.23	8564.23
ψ to+s φ a2*f*s p s*to+t	40	8491.122	8571.122
ψ f*to*t φ a2*f*s p to*s	127	8321.296	8575.296
ψ f*to*t φ a2 p to*s	118	8345.599	8581.599
ψ f*to*t φ s p to*s	118	8347.776	8583.776
ψ f*to*t φ f*s p to*s	122	8340.499	8584.499
ψ f*to*t φ a2*t p to*s	149	8286.553	8584.553
ψ f*to*t φ t p to*s	134	8317.292	8585.292
ψ f*to*t φ a2*f p to*s	122	8347.059	8591.059
ψ f*to*t φ t*s p to*s	154	8286.099	8594.099
ψ f*to*t φ a2*t*s p to*s	189	8219.292	8597.292
ψ f*to*t φ a2*s p to*s	120	8359.345	8599.345
ψ to*t φ a f*s p to*s	155	8290.356	8600.356
ψ to*t φ a2*f*s p to+t	88	8428.296	8604.296
ψ f*to*s φ a2*f*t*s p to*t	229	8150.281	8608.281
ψ to*t φ a2*f*s p to*t	124	8360.998	8608.998
ψ to*t φ a[NL] f*s p to*s	68	8478.552	8614.552
ψ f*to*t φ f p to*s	118	8381.918	8617.918
ψ s*to+t φ a2*s+t p to*s	52	8513.92	8617.92
ψ to*t φ a2*f*s p to+s	71	8478.515	8620.515
ψ f*to*t φ f*t p to*s	169	8284.175	8622.175
ψ to*t φ a2*s p to+s	64	8495.546	8623.546
ψ f*t φ a2*s p to*t	116	8402.963	8634.963
ψ to*t φ a2*s p to*t	116	8408.913	8640.913

ψ *f*to*t* φ *a2*f*t* p *to*s*	216	8226.476	8658.476
ψ *f*to*t* φ *a2*f*t*s* p *to*s*	270	8118.946	8658.946
ψ *to*t* φ *a2*f*s* p *to*	71	8518.116	8660.116
ψ *f*to*t* φ *a2*s* p *to*s*	120	8429.603	8669.603
ψ *f*t* φ *a2*s* p *to*s*	65	8539.804	8669.804
ψ *f*to*t* φ *f*t*s* p *to*s*	221	8230.478	8672.478
ψ *to*s* φ *a2*f*t*s* p *to*t*	245	8189.644	8679.644
ψ *f*to+s* φ *a2*s+t* p *to*s*	35	8610.904	8680.904
ψ *to*t* φ *a2*f*t*s* p *to*s*	252	8178.474	8682.474
ψ *f*to*t* φ *a2*s* p *to*t*	170	8346.479	8686.479
ψ *f*s* φ *a2*f*t*s* p *to*t*	243	8202.871	8688.871
ψ *f*to* φ *a2*f*t*s* p *to*t*	232	8225.226	8689.226
ψ *to* φ *a2*f*t*s* p *to*t*	213	8266.95	8692.95
ψ *to*t*s* φ *a2*f*t*s* p *to*t*	330	8036.201	8696.201
ψ *to*t* φ *a2*f*t*s* p *to*t*	269	8166.217	8704.217
ψ *f* φ *a2*f*t*s* p *to*t*	232	8244.899	8708.899
ψ *f*t* φ *a2*f*t*s* p *to*t*	281	8169.779	8731.779
ψ *f*to*t* φ *a2*f*t*s* p *to*t*	322	8088.625	8732.625
ψ *f*t* φ *a2*f*t*s* p *to*s*	233	8267.092	8733.092
ψ *f*t*s* φ *a2*f*t*s* p *to*t*	335	8068.258	8738.258
ψ *t*s* φ *a2*f*t*s* p *to*t*	262	8229.089	8753.089
ψ *to*t* φ *a2*f*t*s* p *to*	224	8307.633	8755.633
ψ *s* φ *a2*f*t*s* p *to*t*	227	8302.382	8756.382
ψ *f*to*t*s* φ *a2*f*t*s* p *to*t*	409	7939.564	8757.564
ψ *f*to*t*s* φ *a2*f*t*s* p *to*t*s*	444	7887.051	8775.051
ψ *f*to*t*s* φ *a2*f*t*s* p *to*[t+s]*	415	7950.033	8780.033
ψ *f*to*t*s* φ *a2*f*t*s* p *to*s*	387	8006.345	8780.345
ψ *f*to*t*s* φ *a2*f*t*s* p *to*	377	8035.393	8789.393
ψ *s*to+t* φ *f*s+a2* p *to*s*	37	8717.365	8791.365
ψ *t* φ *a2*f*t*s* p *to*t*	256	8280.929	8792.929
ψ *s*to+t* φ *a2*f*s* p *to*s*	40	8714.501	8794.501
ψ *f*to*s* φ *a2*f*s* p *to*s*	30	8734.942	8794.942
ψ *f*to*t*s* φ *a2*f*t*s* p *t*s*	397	8002.435	8796.435
ψ *f*to*t*s* φ *a2*f*t*s* p *t+s*	388	8023.728	8799.728
ψ *to+t* φ *a2*f*s* p *to*s*	38	8727.56	8803.56
ψ *f*to*t*s* φ *a2*f*t*s* p *to+t+s*	396	8014.395	8806.395
ψ *f*to*s* φ *a2*f*t*s* p *to*s*	205	8437.254	8847.254
ψ *f*to*t*s* φ *a2*f*t*s* p *t*	390	8122.763	8902.763
ψ *f*to*t*s* φ *a2*f*t*s* p *s*	371	8333.573	9075.573

Table A2.3. The complete list of models fitted to the 2006 *I. pumilio* data in order of AIC value. Number of estimable parameters (NP), model deviance and Akaike information criterion (AIC). Subscripts – *a2* = age (*NL* indicates negative linear effect, 2 indicates two-age-class effect), *s* = sex, *t* = time. Terms in square brackets indicate weather effects modelled as constraints on time: *MX* = maximum temperature, *MN* = minimum temperature.

Model	NP	Dev	AIC
$\varphi_{a2} p_{t+s}$	28	2015.532	2071.532
$\varphi_{a2+t[MX]} p_{t+s}$	29	2013.709	2071.709
$\varphi_{a2} p_{t}$	27	2018.137	2072.137
$\varphi_{a2+t[MN]} p_{t+s}$	29	2014.231	2072.231
$\varphi_{a2+t[MX]} p_{t}$	28	2016.403	2072.403
$\varphi_{a2+s} p_{t+s}$	29	2015.487	2073.487
$\varphi_{a2+t[MX]+s} p_{t+s}$	30	2013.565	2073.565
$\varphi_{a2*s} p_{t+s}$	30	2013.661	2073.661
$\varphi_{a2+s} p_{t}$	28	2017.896	2073.896
$\varphi_{a2*s} p_{t}$	29	2016.691	2074.691
$\varphi_{a2+t} p_{t}$	51	1983.027	2085.027
$\varphi_{a2+t} p_{t+s}$	52	1981.419	2085.419
$\varphi_{s} p_{t}$	27	2031.739	2085.739
$\varphi_{a2+t+s} p_{t}$	52	1982.331	2086.331
$\varphi_{a2*s+t} p_{t}$	53	1981.284	2087.284
$\varphi_{t} p_{t}$	48	1993.768	2089.768
$\varphi_{t+s} p_{t}$	51	1992.637	2094.637
$\varphi_{a2} p_{t*s}$	52	1993.379	2097.379
$\varphi_{a2*t} p_{t}$	71	1959.837	2101.837
$\varphi_{a} p_{t}$	50	2003.112	2103.112
$\varphi_{a+s} p_{t}$	51	2002.851	2104.851
$\varphi_{a2*t+s} p_{t}$	74	1958.515	2106.515
$\varphi_{a2+t*s} p_{t}$	76	1960.434	2112.434
$\varphi_{t*s} p_{t}$	73	1972.353	2118.353
$\varphi_{a\,s} p_{t}$	75	1981.953	2131.953
$\varphi_{a2+t[MX]} p_{t[MX]+s}$	6	2141.004	2153.004
$\varphi_{a2} p_{t[MX]+s}$	5	2145.325	2155.325
$\varphi_{a2*t*s} p_{t}$	117	1922.135	2156.135
$\varphi_{a2*t*s} p_{t+s}$	118	1920.480	2156.480
$\varphi_{a2+t[MX]} p_{s}$	5	2148.937	2158.937
$\varphi_{a2} p_{t[MN]+s}$	5	2149.116	2159.116
$\varphi_{a2} p_{s}$	4	2151.948	2159.948
$\varphi_{a2*t*s} p_{t*s}$	140	1898.948	2178.948
$\varphi_{a2*t*s} p_{s}$	96	2041.197	2233.197
$\varphi_{a2*t*s} p_{i}$	95	2044.024	2234.024
$\varphi_{a[NL]} p_{t+s}$	27	2208.411	2262.411
$\varphi_{a2*t[MX]} p_{t+s}$	28	2570.552	2626.552
$\varphi_{t[MX]} p_{t+s}$	27	2592.142	2646.142

Table A2.4. The complete list of models fitted to the *I. pumilio* legs data in order of AIC value. Number of estimable parameters (NP), model deviance and Akaike information criterion (AIC). Subscripts – s = sex, l = leg removal, "." = no effects.

Model	NP	Deviance	AIC
$\varphi_{.}p_{.}$	2	1066.589	1070.589
$\varphi_{.}p_{s}$	3	1066.425	1072.425
$\varphi_{.}p_{l}$	3	1066.466	1072.466
$\varphi_{l}p_{.}$	3	1066.537	1072.537
$\varphi_{s}p_{.}$	3	1066.575	1072.575
$\varphi_{l}p_{s}$	4	1066.374	1074.374
$\varphi_{s}p_{s}$	4	1066.423	1074.423
$\varphi_{l}p_{l}$	4	1066.439	1074.439
$\varphi_{s*l}p_{.}$	5	1066.515	1076.515
$\varphi_{.}p_{t}$	25	1028.979	1078.979
$\varphi_{.}p_{t[NL]}$	2	1076.821	1080.821
$\varphi_{t}p_{.}$	25	1043.935	1093.935
$\varphi_{t*l}p_{.}$	49	1021.789	1119.789
$\varphi_{t*s}p_{.}$	49	1028.167	1126.167
$\varphi_{t*s*l}p_{.}$	96	993.820	1185.820
$\varphi_{t*s*l}p_{s}$	97	993.628	1187.628
$\varphi_{t*s*l}p_{l}$	97	993.726	1187.726
$\varphi_{t*s*l}p_{t}$	118	952.241	1188.241
$\varphi_{t*s*l}p_{s*l}$	99	993.520	1191.520
$\varphi_{t*s*l}p_{t*s}$	140	924.831	1204.831
$\varphi_{t*s*l}p_{t*s*l}$	169	867.396	1205.396
$\varphi_{t*s*l}p_{t*l}$	140	934.654	1214.654
$\varphi_{t[NL]}p_{.}$	2	1339.815	1343.815

Table A2.5. The complete list of models fitted to the Itchen *Coenagrion mercuriale* data in order of AIC value. Number of estimable parameters (NP), model deviance and Akaike information criterion (AIC). Subscripts – $a2$ = two-age-class effect, l = location/site, m = trap-dependence effect, s - sex, t - time.

Model	NP	Deviance	AIC
$\varphi_{t+l}p_{t*l+s}$	79	20709.66	13693.73
$\varphi_{t+s+l}p_{t*l+s}$	80	20709.63	13695.7
$\varphi_{t}p_{t*l+s}$	77	20721.5	13697.47
$\varphi_{t+s*l}p_{t*l+s}$	82	20708.73	13699.12
$\varphi_{t*s+l}p_{t*l+s}$	98	20688.73	13718.05
$\varphi_{t+l}p_{t*s*l}$	124	20640.86	13738.76
$\varphi_{t+s+l}p_{t*s*l}$	125	20639.84	13740.09
$\varphi_{t}p_{t*s*l}$	122	20652.42	13742.31
$\varphi_{t+s*l}p_{t*s*l}$	127	20637.86	13742.79
$\varphi_{t+s*l}p_{t*l}$	127	20637.86	13742.8
$\varphi_{t+s}p_{t*s*l}$	123	20650.97	13743.37

$\varphi_{t+s+1}p_{t*l}$	79	20808.98	13758.64
$\varphi_{t*s}p_{t*l}$	95	20772.36	13766.71
$\varphi_{t*l}p_{t*s*l}$	150	20606.07	13768.02
$\varphi_{t*s}p_{t*s*l}$	141	20634.97	13768.91
$\varphi_{t+1}p_{t+s+1}$	45	20944.71	13779.35
$\varphi_{t+s+1}p_{t+s+1}$	46	20944.59	13781.28
$\varphi_{t+1}p_{t*s+1}$	47	20943.46	13782.54
$\varphi_{t}p_{t+s+1}$	43	20961.93	13786.6
$\varphi_{t+1}p_{t+s*l}$	64	20913.26	13796.8
$\varphi_{t}p_{t*s+1}$	62	20930.37	13803.98
$\varphi_{t*s*l}p_{t*s*l}$	181	20573.56	13808.77
$\varphi_{t+1}p_{t*l}$	75	20930.47	13830.04
$\varphi_{t}p_{t*l}$	73	20943.34	13834.46
$\varphi_{t*l+s}p_{t*l+s}$	107	20857.02	13846.04
$\varphi_{t+s+1}p_{t+1}$	45	21049.29	13847.7
$\varphi_{t*l}p_{t*l}$	98	20897.82	13854.71
$\varphi_{t*l}p_{t*s}$	95	20971.97	13897.17
$\varphi_{t+1}p_{t+s}$	43	21138.87	13902.26
$\varphi_{t+s+1}p_{t+s}$	44	21137.87	13903.6
$\varphi_{t}p_{t+s}$	41	21169.02	13917.96
$\varphi_{t+1}p_{t*s}$	62	21107.56	13919.79
$\varphi_{t+s+1}p_{t*s}$	63	21105.29	13920.31
$\varphi_{t+1}p_{t+1}$	44	21171.05	13925.29
$\varphi_{t}p_{t+1}$	42	21188.87	13932.93
$\varphi_{t}p_{t*s}$	59	21137.47	13933.34
$\varphi_{t*s}p_{t*s}$	76	21118.45	13954.91
$\varphi_{a2*t+1}p_{t*l+s}$	81	21229.01	14037.17
$\varphi_{a2*l}p_{t*s*l}$	107	21204.22	14072.97
$\varphi_{a2}p_{t*s*l}$	106	21207.56	14073.15
$\varphi_{a2*s*l}p_{t*s*l}$	109	21199.62	14073.96
$\varphi_{a2*t}p_{t*s*l}$	124	21153.93	14074.1
$\varphi_{s}p_{t*s*l}$	105	21233.09	14087.84
$\varphi_{a2*t*s}p_{t*s*l}$	136	21139.51	14088.67
$\varphi_{1}p_{t*s*l}$	106	21232.7	14089.58
$\varphi_{s*l}p_{t*s*l}$	109	21223.6	14089.63
$\varphi_{s+1}p_{t*s*l}$	107	21230.61	14090.22
$\varphi_{a2*t}p_{t*s*l}$	147	21130.28	14104.64
$\varphi_{a2*t*s*l}p_{t*s*l}$	151	21124.25	14108.7
$\varphi_{a2*t*s*l}p_{t*l}$	117	21244.91	14119.56
$\varphi_{a2*t*s*l}p_{t*s}$	106	21545.44	14293.99
$\varphi_{a2*t*s*l}p_{t}$	88	21626.87	14311.21
$\varphi_{t+1}p_{m*t*l+s}$	80	22851.08	15095.35
$\varphi_{t}p_{s+1}$	24	23310.76	15283.79
$\varphi_{a2*t*s*l}p_{m*t*s}$	98	24154.81	15983.46
$\varphi_{a2*t*s*l}p_{m*t*l}$	112	24125.75	15992.46
$\varphi_{a2[15]*t*s*l}p_{m[15]*t*s*l}$	116	24114.55	15993.14
$\varphi_{a2*t*s*l}p_{m*t}$	86	24249.1	16021.08
$\varphi_{a2*t*s*l}p_{l}$	71	24515.23	16165.03
$\varphi_{a2*t*s*l}p_{m*s}$	69	24620.89	16230.09
$\varphi_{a2*t*s*l}p_{m*s*l}$	70	24618.54	16230.55

$\varphi_{*a2*t*s*l}p_{m*l}$	71	24621.49	16234.48
$\varphi_{a2*t*s*l}p_{m}$	64	24668.39	16251.13
$\varphi_{a2*t*s*l}p_{s}$	70	24679.63	16270.48

Table A2.6. The complete list of models fitted to the Beaulieu *Coenagrion mercuriale* data in order of AIC value. Number of estimable parameters (NP), model deviance and Akaike information criterion (AIC). Subscripts – *a2* = two-age-class effect, *l* = location/site, *m* = trap-dependence effect, *s* - sex, *t* - time.

Model	NP	Deviance	AIC
$\varphi_{t+s+l}p_{t*l+s}$	84	34153.531	31501.515
$\varphi_{a2*t+l}p_{t*l+s}$	85	34153.270	31503.275
$\varphi_{t+l}p_{t*l+s}$	83	34157.821	31503.451
$\varphi_{t+s+l}p_{t*[l+s]}$	99	34132.610	31512.321
$\varphi_{a2*t+s}p_{t*l+s}$	83	34172.914	31517.297
$\varphi_{t*l+s}p_{t*l+s}$	125	34082.919	31518.733
$\varphi_{a2+t*l}p_{t*l+s}$	126	34084.174	31521.885
$\varphi_{t}p_{t*l+s}$	80	34203.610	31539.459
$\varphi_{a2*t}p_{t+s+l}$	38	34323.430	31565.385
$\varphi_{a2*t}p_{t*s*l}$	137	34116.417	31573.466
$\varphi_{t*l}p_{t*s*l}$	176	34035.648	31577.365
$\varphi_{s+l}p_{t*l+s}$	69	34271.065	31579.344
$\varphi_{a2*t*l}p_{t*s*l}$	177	34046.549	31589.366
$\varphi_{a2*t*s}p_{t*s*l}$	152	34101.648	31589.916
$\varphi_{t}p_{t*s*l}$	135	34152.168	31602.265
$\varphi_{t*s}p_{t*s*l}$	150	34136.792	31618.158
$\varphi_{a2*t*s*l}p_{t*s*l}$	210	34006.914	31619.004
$\varphi_{t*s*l}p_{t*s*l}$	223	33986.549	31626.321
$\varphi_{s*l}p_{t*s*l}$	128	34212.248	31643.384
$\varphi_{l}p_{t*s*l}$	124	34225.380	31647.432
$\varphi_{a2*l}p_{t*s*l}$	126	34224.942	31651.029
$\varphi_{a2*s}p_{t*s*l}$	124	34240.175	31661.004
$\varphi_{a2}p_{t*s*l}$	123	34243.984	31662.499
$\varphi_{s}p_{t*s*l}$	122	34259.260	31674.514
$\varphi_{s}p_{t*s*l}$	122	34259.260	31674.514
$\varphi_{t+s+l}p_{t+s}$	37	34472.479	31700.127
$\varphi_{t+s+l}p_{t+s}$	37	34472.479	31700.127
$\varphi_{t+s+l}p_{t*s}$	52	34448.971	31708.560
$\varphi_{t+s+l}p_{t.s}$	52	34448.971	31708.560
$\varphi_{t+l}p_{t+s+l}$	39	34488.087	31718.447
$\varphi_{t*l}p_{t*s}$	94	34371.981	31721.927
$\varphi_{t*l}p_{t*s}$	94	34371.981	31721.927
$\varphi_{t+s+l}p_{t*l}$	83	34406.889	31731.953
$\varphi_{t+s+l}p_{t*l}$	83	34406.889	31731.953
$\varphi_{a2*t*l}p_{t*s}$	97	34382.895	31737.940
$\varphi_{a2*t*l}p_{t*s}$	97	34382.895	31737.940
$\varphi_{a2*t*s*l}p_{t*s}$	137	34340.357	31778.915
$\varphi_{a2*t*s*l}p_{t*s}$	137	34340.357	31778.915

$\varphi_{a2*t*s*l}p_{t*l}$	163	34284.546	31779.712
$\varphi_{a2*t*s*l}p_{t*l}$	163	34284.546	31779.712
$\varphi_{a2*t}p_{t+s}$	35	34635.457	31845.649
$\varphi_{a2*t}p_{t*s}$	50	34610.089	31852.375
$\varphi_{a2*t}p_{t*s}$	50	34610.089	31852.375
$\varphi_{a2*t*s*l}p_{t}$	122	34558.800	31949.321
$\varphi_{a2*t*s*l}p_{t}$	122	34558.800	31949.321
$\varphi_{a2*t}p_{t*l}$	80	34719.558	32012.805
$\varphi_{a2*t}p_{t*l}$	80	34719.558	32012.805
$\varphi_{a2*t*l}p_{t*l}$	121	34634.310	32016.597
$\varphi_{a2*t*l}p_{t*l}$	121	34634.310	32016.597
$\varphi_{t*l}p_{t*l}$	118	34677.909	32050.595
$\varphi_{t*l*}p_{t*l}$	118	34677.909	32050.595
$\varphi_{a2*t}p_{t+l}$	37	34870.951	32065.698
$\varphi_{a2*t*s*l}p_{m*t*s}$	139	35041.919	32426.550
$\varphi_{a2*t*s*l}p_{m*t*s}$	139	35041.919	32426.550
$\varphi_{a2*t*s*l}p_{m*t*s*l}$	180	34956.422	32430.112
$\varphi_{a2*t*s*l}p_{m*t*s*l}$	180	34956.422	32430.112
$\varphi_{a2*t}p_{m*t*s*l}$	108	35164.384	32476.903
$\varphi_{a2*t}p_{m*t*s*l}$	108	35164.384	32476.903
$\varphi_{a2*t*s*l}p_{m*t*l}$	163	35082.452	32511.736
$\varphi_{a2*t*s*l}p_{m*t*l}$	163	35082.452	32511.736
$\varphi_{t+s+l}p_{s+l}$	25	35412.002	32538.075
$\varphi_{t+s+l}p_{l+s}$	25	35412.002	32538.075
$\varphi_{t+s+l}p_{l+s}$	25	35412.002	32538.075
$\varphi_{a2*t*s*l}p_{m*t}$	124	35320.587	32652.208
$\varphi_{a2*t*s*l}p_{m*t}$	124	35320.587	32652.208
$\varphi_{a2*t}p_{s+l}$	23	35615.003	32720.315
$\varphi_{a2*t*s*l}p_{m*s*l}$	103	35532.018	32804.182
$\varphi_{a2*t*s*l}p_{m*s*l}$	103	35532.018	32804.182
$\varphi_{a2*t*s*l}p_{s*l}$	114	35515.193	32810.746
$\varphi_{a2*t*s*l}p_{s*l}$	114	35515.193	32810.746
$\varphi_{a2*t*s*l}p_{m*s}$	110	35544.514	32829.646
$\varphi_{a2*t*s*l}p_{m*s}$	110	35544.514	32829.646
$\varphi_{a2*t*s*l}p_{m*l}$	112	35626.069	32908.467
$\varphi_{a2*t*s*l}p_{m*l}$	112	35626.069	32908.467
$\varphi_{a2*t*s*l}p_{s}$	108	35651.653	32923.938
$\varphi_{a2*t*s*l}p_{s}$	108	35651.653	32923.938
$\varphi_{a2*t*s*l}p_{l}$	110	35741.926	33010.758
$\varphi_{a2*t*s*l}p_{l}$	110	35741.926	33010.758
$\varphi_{a2*t*s*l}p_{m}$	108	35795.152	33055.589
$\varphi_{a2*t*s*l}p_{m}$	108	35795.152	33055.589
$\varphi_{s+l}p_{s+l}$	10	36657.435	33650.674
$\varphi_{s+l}p_{s+l}$	10	36657.435	33650.674